UNIVERSITY OF ALBERTA

HETEROSIS, OUTCROSSING, AND SPOROPHYTIC INCOMPATIBILITY STUDIES IN RAPE (*BRASSICA NAPUS* L.)

BY

LANCE JOHN LEWIS

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF GENETICS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled HETEROSIS, OUTCROSSING, AND SPOROPHYTIC INCOMPATIBILITY STUDIES IN RAPE (*BRASSICA NAPUS* L.) submitted by LANCE LEWIS in partial fulfillment of the requirements for the degree of Master of Science in Genetics.

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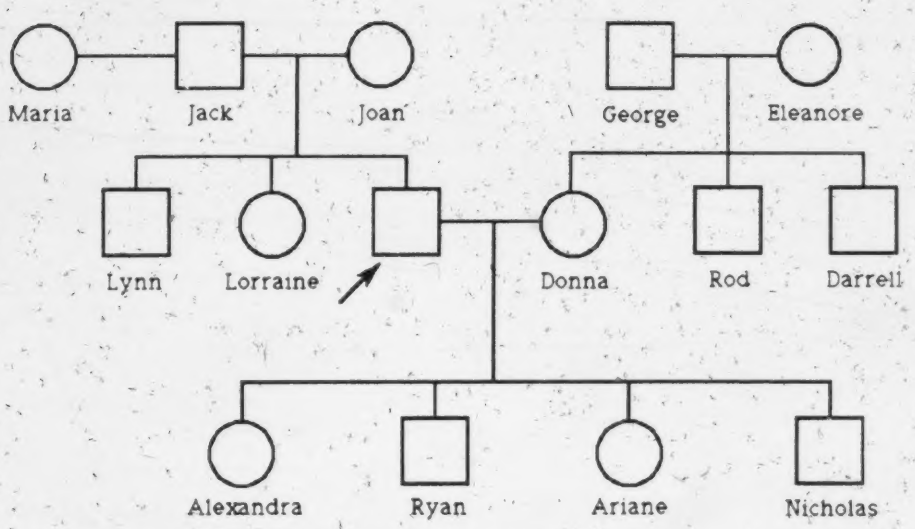
Date: June 22 1990

DEDICATION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ABSTRACT

Seven lines and 5 varieties of summer oilseed rape (*Brassica napus* L.) were crossed in diallel and hybrids tested for combining ability effects. General, specific, and reciprocal combining abilities were significant for all agronomic traits measured supporting earlier reports of superior F_1 hybrid performance in rape.

Interplant outcrossing rates were determined for 16 lines of *B. napus* using erucic acid (cis-13-docosenoic) as a marker. The rates of outcrossing for each line was compared with previously established rates for parents using rank correlation tests. Correlations were marginally significant indicating that a genetic component could be responsible for the various outcrossing rates observed. The grand mean outcrossing rate at Beaverlodge, Alberta in 1988 was 55.3 percent.

Presence of 'residual' sporophytic incompatibility (RSI) in domestic rape was tested by reciprocal cross pollination using mixed pollen from both parents, then screening progeny to determine outcrossing rates using a recessive pale-flower mutant-high erucic acid line and wild type flower colour-low erucic acid parents. One Westar plant was identified with potential RSI, and sporophytic incompatible (SI) controls were successfully identified.

Introgression of SI into sporophytic compatible (SC) summer rape was attempted using SI *B. oleracea*, *B. campestris*, and *B. napus* ssp. *rapifera* (Swedes). Introgression was easily accomplished using the latter two species, but unsuccessful with *B. oleracea*. Allelic relationships of 6 plants with introgressed Swede SI alleles was determined by reciprocally chain crossing progeny and screening using a pollen tube assay from which 4 alleles were identified.

Two dominant SI alleles were introgressed into 5 lines of SC rape. Each line was backcrossed for two generations and SI plants selected by pollen tube or seed set assays. Segregating SI and SC lines were increased and grown under field conditions at three sites in Alberta. No significant differences in performance were observed between SC and SI lines for any agronomic trait measured suggesting that their is no 'biological cost' associated with introgressed sporophytic incompatibility in rape.

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LIST OF ABBREVIATIONS

A-lines	male sterile (cytoplasmic male sterile system)
B-lines	maintainers (cytoplasmic male sterile system)
CMS	cytoplasmic male sterility
cr	cross-pollinated plant
g.c.a.	general combing ability
GC	gas chromatography
GI	gametophytic incompatibility
GMS	genic male sterility
NMR	nuclear magnetic resonance
PC	paper chromatography
PSC	pseudo-self compatibility
R-lines	restorers (cytoplasmic male sterile system)
r.c.a.	reciprocal combining ability
RI	residual incompatibility
s.c.a.	specific combining ability
SC	self-compatibility (sporophytic compatible)
SI	sporophytic incompatibility
sl	self-pollinated plant

1.0 Introduction

Crop *Brassicas* are grown as root or vegetables, sources of oil or condiments, and as fodder. The small, round seeds of the two rapeseed species, *Brassica napus* L. (rape) and *Brassica campestris* L. (turnip rape) contain 40 to 44% oil on a dry-weight basis and produce an oil-free meal with 38-41% protein (Downey and Rakow, 1987). Oils processed from "canola" quality rapeseed (rapeseed varieties with < 2.0% erucic acid of the total fatty acids in the oil and < 30 μmoles g^{-1} glucosinolates of air dry, oil-free meal; Seeds Act, 1989) are used in shortening, margarine and salad oils. Oils high in erucic acid have an industrial market such as lubricants for metal surfaces, plasticizers and for synthesis of erucamide which is used in the manufacture of plastic films. The high protein meal is suitable for supplementing animal diets and, in some Asian countries, is used as an organic fertilizer. *B. napus* and *B. campestris* are currently the only canola quality species cultivated, but breeding advances may soon enable commercial production of canola quality *B. juncea* (Canola Digest, 1988).

Canola is of considerable importance to the Canadian economy. Commercial production of rapeseed in Canada was initiated during the Second World War to provide an essential lubricant for naval and merchant ships since European and Asiatic rapeseed supplies were disrupted. The demand for oil resulted in production of 1,200 kg of *B. napus* by Agriculture Canada Experimental Farms and Stations in 1942 (Kondra, 1985). Since the Second World War, canola has become the leading Canadian oilseed crop in terms of both its volume and value of production. Canola, and soybean to a lesser extent, have enabled extensive substitution of imported oilseeds and products and have generated a major Canadian food processing industry (Veeman, 1985). While the global production of oilseeds and protein meal was

dominated by soybean in 1986-87, canola accounted for 10% of world oilseed production (Agriculture Canada, Fats and Oils. Annual review, 1988). When processed, canola contributed 10.1% protein meals and 14.1% edible vegetable oils in the 1986-1987 calendar year while soybean contributed 60.9% and 31.4% respectively. Canada has produced an average 18.4% of the world rapeseed production from 1982 to 1986 and is the second largest producer next to China. In 1989, Canadians seeded 2.907 million hectares of canola with an average yield of 1050 kg ha^{-1} (Statistics Canada, 1989). With an approximate value of \$263 $tonne^{-1}$ (Grain Statistics Weekly, 1990) canola had a value of \$803 million in gross farm receipts.

The economic value of Brassica oil and meal has stimulated research in the development of hybrids. Recent studies have indicated that commercial production of hybrid F_1 *B. napus* may be possible with an adequate levels of heterosis, pollen transfer, and pollen control (Schuster and Michael, 1976; Shiga, 1976; Campbell and Kondra, 1978; Morice, 1978; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985). The objective of this thesis is to investigate sporophytic incompatibility (SI) in summer rape [*Brassica napus* L. ssp. *oleifera* forma *annua* (Metzg.)] as a method of pollen control. Research is directed to: a) determine heterosis and combining ability estimates; b) assess the degree of natural outcrossing of *B. napus* at one site in western Alberta; c) screen domestic varieties and lines to establish if residual SI is present in these populations; d) introgress S-alleles into *B. napus* by using intraspecific and interspecific crosses; and e) assess the development, heritability, and agronomic performance of closely related self-compatible (SC) and SI *B. napus* lines.

2.0 Literature review

2.1 Nomenclature

Crop *Brassicas* belong to the Family Brassicaceae (syn. Cruciferae). Morinaga (1929) gave cytological evidence that the high chromosome number *Brassica* species *B. juncea* (L.) Czern. and Coss. (2n = 36, AABB), *B. napus* L. (2n = 38, AACC) and *B. carinata* A. Braun (2n = 34, BBCC) were amphidiploid (allotetraploid) derivatives of the low chromosome number species *B. campestris* L. (2n = 20, AA), *B. nigra* (L.) Koch (2n = 16, BB), and *B. oleracea* L. (2n = 18, CC). U (1935) confirmed this hypothesis by synthesizing artificial *B. napus* by doubling the chromosome number of amphihaploids derived from crosses between *B. oleracea* and *B. campestris* (Figure 2.1). Although the phylogenetic origins of the *Brassica* diploid species are not well understood, they are believed to be secondary polyploids as well (Röbbelen, 1960). On the basis of autosyndetic pairing and chromosome morphology, Catcheside (1934) and Röbbelen (1960) suggested that the elementary monogenomic species were derived from an extinct species with a basic chromosome number of x = 6. Most cytogenetic evidence supports this hypothesis (Prakish and Hinata, 1980; Mitushima, U, 1980; Armstrong and Keller, 1981, 1982) as does enzyme electrophoresis studies (Chen *et al.*, 1989) although the genome number in *Brassica* ranges from x = 7 to x = 19 (Prakash and Hinata, 1980) and existence of past or present x = 6 *Brassica* species has not been reported (Quiros *et al.* 1988).

2.2 Flower morphology of *Brassicas*

Brassica flowers are bisexual, and radially symmetrical with four sepals alternating with four petals, the latter giving the resemblance of a cross (hence

the name Cruciferae). Petals vary in colour from dark yellow to white but are usually pale yellow. Flowering is indeterminate and the inflorescence racemose with the lowest bud on the main raceme flowering first, followed by the lower flowers on lateral branches three to five days later. The stigma is receptive for approximately three days after anthesis. The androecium is tetradynamous consisting of two short lateral, introse stamens and four long median stamens that develop from introrse in the bud to extrorse as the flower opens (Downey and Rakow, 1987). Ovaries are hypogynous with a "false" partition, apparently from the middle of one parietal placenta to the middle of the other (Benson, 1959) and the fruits are glabrous siliques. The silique consists of two carpels separated by the replum (false septum). At maturity, the two carpels easily split from the false septum thereby shedding 15-40 seeds silique^{-1} (Bengtsson *et al.*, 1972; see Gómez-Campo, 1980, for a detailed discussion of morphology).

Both annual (spring or summer) and biennial (winter) forms of *B. napus* and *B. campestris* exist but in *B. juncea* only the summer form has evolved. The current emphasis of rapeseed breeding programs in Canada has been the development of superior cultivars of summer *B. napus* (rape) and to a lesser extent, *B. campestris* (turnip rape) due primarily to the higher yield potential of rape (Downey and Rakow, 1987). Of the rapeseed species, only *B. napus* will be considered further.

2.3 Outcrossing rate of *B. napus*

Rape is predominantly a self-pollinating species (Olsson, 1960b) which exhibits outcrossing rates of 20 to 40% under field conditions (Olsson, 1952; Persson, 1956; Olsson 1960b; Rakow and Woods, 1987; Banks, 1988). Using recessive petal colour markers Olsson (1952) reported 34.6% outcrossing in

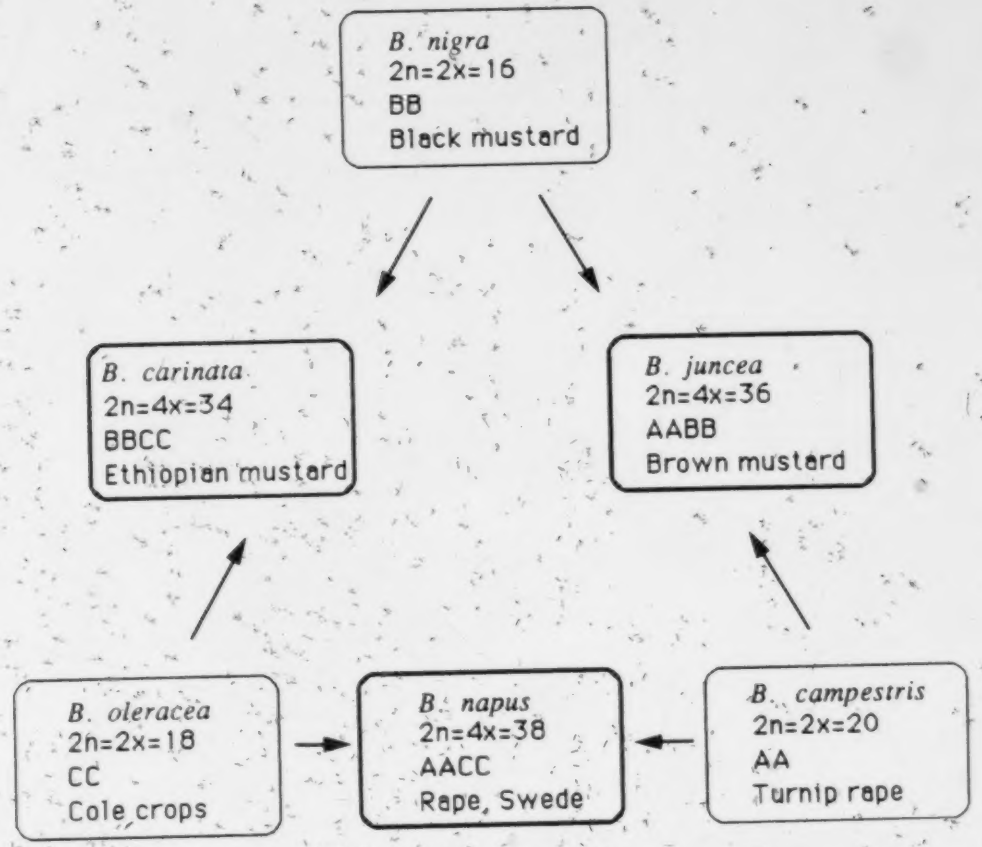


Figure 2.1 Genomic relationship of *Brassica* species (modified from U, 1935).

two cultivars of winter rape. Persson (1956), also using a petal colour marker, reported a mean interplant outcrossing within plots of 36% in summer rape and 27-30% for winter rape. Olsson (1960b) reviewed outcrossing rates for winter and summer rape and found a mean of 35.9% outcrossing. Rives (1957) suggested that Olsson's early outcrossing estimates were inaccurate since the recessive pale-flower segregants were earlier flowering than dominant yellow-flower wild types, weaker than the surrounding crop, and sometimes difficult to distinguish from the wild types. Also, Faulkner (1971) found that honey bees (*Apis mellifera* L.) worked in a highly selective fashion that distinguished field planted inbred lines of Brussels sprouts (*B. oleracea*). Bee visits to inbreds had a mean selfing to crossing ratio of 23:1 and 33:1 for plants differing in flower colour and plant height respectively (Faulkner, 1976) thus encouraging selfing and sibbing (Faulkner 1971, 1974, 1976). Stanton *et al.*, (1989) reported preferential insect visitation to specific polymorphic colour variants in wild radish (*Raphanus raphanistrum*) corroborating Faulkner's observations in another Brassicaceae (for further references, see Stanton *et al.*, 1989). Since honey bees are an important pollination vector for rapeseed (Williams, 1978; Holm *et al.*, 1985; Sihag, 1985, 1986) the bee behaviour studies of Faulkner seriously undermine early attempts to determine outcrossing rates using flower colour markers.

More recent attempts to determine rates of outcrossing have exploited the biochemical marker erucic acid. Erucic (cis-13-docosenoic) acid, a mono-ethanoic acid with 22 carbon atoms ($C_{22:1}$), occurs extensively in seed oils of Brassicaceae. In *Brassica* species this long chain fatty acid is under the control of the embryonic genotype, rather than the maternal plant, permitting genetic analysis of populations based on single seeds or half seeds of F_1 plants (Downey and Harvey, 1963). Erucic acid in *B. napus* is governed by the

interaction of two independently inherited genes (Harvey and Downey, 1964; Kondra and Stefansson, 1965; Chen and Heneen, 1989a; Siebel and Pauls, 1989) whose functional alleles predominantly act in an additive manner (Downey and Craig, 1964; Dorrell and Downey, 1964; Harvey and Downey, 1964; Stefansson and Hougen, 1964; Kondra and Stefannson, 1965; Chen *et al.*, 1988a) although partial dominance for high erucic acid content has been observed (Jönsson, 1977a; Chen and Heneen, 1989a). Harvey and Downey (1964) proposed that the two genes were derived from *B. campestris* (AA) and *B. oleracea* (CC), the progenetors of *B. napus* (AACC). This proposal has been subsequently confirmed (Fernandez-Escobar *et al.*, 1988; Chen and Heneen, 1989a). The initial concept of erucic acid gene control suggested that each functional allele contributes approximately 10% erucic acid content (Harvey and Downey, 1964). Krzymanski and Downey (1969) reported another allele controlling erucic acid synthesis, but suggested that all 5 alleles were active at a single locus. Adopting the symbolism of Krzymanski and Downey (1969), the 5 alleles and corresponding (%) erucic acid synthesis for each is: e (< 1%), E^a (~ 10%), E^b (~ 15%), E^c (~ 30%), and E^d (~ 3.5%). Anand and Downey (1981) confirmed that E^a and E^d are true alleles in the C-genome of *B. napus*, but when considering environmental factors, modifying genes, genetic background, and ploidy level, no direct evidence is available to support e, E^b, and E^c as true alleles (Anand and Downey, 1981; Chen and Heneen, 1989a). Jönsson (1977ab) suggested multiple alleles active at both loci could account for erucic acid values from 0 to above 60% of total fatty acids. Calhoun *et al.* (1975) reported a Sarson (*B. campestris*) accession from India with 65.8% erucic acid. The upper limit for erucic acid is not expected to be significantly higher than this accession (the maximum hypothetical value is 67 mole percent) since

esterification of $C_{22:1}$ is restricted to the 1 and 3 atoms of the glycerol (Tallent, 1972).

Erucic acid is superior to visible character markers such as flower colour since it is thought that pollinators cannot discriminate between flowers derived from seed with different fatty acid compositions. In a European cultivar trial, Hühn and Rakow (1979) used the erucic acid marker and reported outcrossing rates of 5-15% between plots in *B. napus* winter rape. A mean interplant outcrossing rate of 21.8% for 4 lines and one cultivar of summer rape tested over 3 years was reported by Rakow and Woods (1987) under Saskatchewan prairie conditions. Using 4 low erucic acid cultivars over 3 years in Southern Ontario, Banks (1988) found a mean interplant outcrossing rate of 36% for geometric planting and 41% for random planting.

2.4 Inbreeding Depression

The majority of higher plants suffer a reduction in vigor and fecundity upon inbreeding, but self-pollinating species and a few outcrossing species are tolerant of inbreeding (Allard, 1964). In the *Brassica* genus, the species *B. oleracea*, *B. campestris* and *B. nigra* are allogamous and suffer significant inbreeding depression while their allotetraploid derivatives (*B. napus* , *B. carinata*, and *B. juncea*) are predominantly autogamous and thereby more tolerant of inbreeding. Schuster (1969) reported a significant yield reduction in inbred lines of the winter rape variety Giessener Hohenraps from I_1 to I_4. Schuster and Michael (1976) later confirmed significant inbreeding depression for yield, height and oil concentration testing 1549 I_1, 388 I_2, 187 I_3 and 97 I_4 lines from Giessener Hohenraps. Meng and Liu (1986) found that oil concentration decreased upon inbreeding.

Falconer (1981) indicated that inbreeding depression affecting a character was based on directional dominance (i.e. when all dominant alleles affect the trait in one direction), gene frequency, and the number of segregating alleles. Inbreeding depression occurs in characters determined primarily by recessive alleles at various loci. However, a significant increase in performance of an inbred over a source parent is possible by additive genetic effects. Brandle and McVetty (1989b) used this concept of inbreeding depression to interpret data from inbred summer rape derived from the seven cultivars: Westar, Regent, Lergo, Marnoo, Ariel, Karat, and R83-11 tested at S_4 and S_5 for 1986 and 1987 respectively. These authors found a mixed response to flowering, maturity, height, yield, oil, and protein where individual lines for each trait were identified that exceeded or equalled the means of the source cultivar. Some inbred lines exceeded the source cultivar suggesting that rape cultivars were from genetically heterogeneous populations where maximum performance is not based on maintained heterozygosity or genetic heterogeneity *per se*. Rather, additive genetic variance can be selected for in inbred lines with an increased frequency of homozygous alleles (Brandle and McVetty, 1989b).

2.5 Hybrid development

The successful development of hybrid rapeseed requires an adequate level of heterosis, sufficient pollen transfer from pollen to seed parents, and an efficient mechanism of pollination control (Grant and Beversdorf, 1985). Each of these three components will be discussed in turn.

2.5.1 Heterosis

Heterosis, or hybrid vigour, can be defined as the superiority of heterozygous genotypes in comparison with corresponding parental

homozygotes and expressed as increased size, vigour, yield, and resistance to climatic extremes or diseases. Hybrids may be derived from crosses involving parental clones, inbred lines, varieties, or breeding populations. Several methods of calculating heterosis have been reported, two of which are mid-parent (classical heterosis) and high-parent (heterobeltiosis, better parent) heterosis. Mid-parent heterosis is defined as: [(Value of F_1 - Value of Mid-parent)/Value of Mid-parent] x 100, where Value of mid-parent is: (Value of Parent 1 + Value of Parent 2)/2 (Falconer, 1981); and high-parent heterosis is defined as: [(Value of F_1 - Value of Better Parent)/Value of Better Parent] x 100 (Fonseca and Patterson, 1968). The hypothesis most widely accepted as being the genetic basis of heterosis, and the conversely related phenomenon of inbreeding depression, is the dominance (dominance of linked genes) hypothesis (Bruce, 1910). The dominance hypothesis suggests that hybrids are superior to parental components since the cumulative effects of heterozygous alleles at numerous loci mask otherwise deleterious recessive alleles present in the parents. Also widely supported is the 'over-dominance hypothesis' (super-dominance, cumulative action of divergent alleles, single-gene heterosis) which suggests that, at some loci, the heterozygote is superior to either homozygote and hybrid vigour increases proportional to heterosis (Hull, 1945). Literature supporting both theories (reviewed by Allard, 1964; Briggs and Knowles, 1967) suggest that dominance and over-dominance both occur and are probably not mutually exclusive phenomena.

Superior performance of F_1 hybrid oilseed rape has been reported for many years. Wagner (1954) developed inbred lines (lg) from the variety Lembke and placed the inbreds in a diallel cross. The F_1's showed significant mid-parent heterosis for yield, but none of the hybrids were superior to the original variety. Rives (1957) reported significant heterosis for yield in some rape crosses.

Schelkudenko (1972) reported an average seed yield gain of 12.8% when testing 112 intervarietal hybrids of winter rape. Shiga (1976) reported 108 of 131 hybrids derived from crosses to a male sterile line yielded as well, or better than the pollen parent. Schuster and Michael (1976) showed heterosis in yield and height for hand crossed hybrids, but little heterosis for oil content. Campbell and Kondra (1978) found significant heterosis for yield and yield components in single plants of hybrid crosses from the Canadian oilseed rape cultivars Nugget, Oro and Target. Morice (1979) reported hybrid rape with 36% seed gain in hand crosses between the French variety Primor and other lines. Significant mid- and high-parent heterosis for seed yield and yield components in 11 male-sterile derived hybrids and 8 handcrossed intervarietal hybrids has been reported by Guan *et al.* (1980). Sernyk and Stefansson (1983) generated intervarietal hybrids of summer oilseed rape by crossing seven cultivars of diverse origin with the Canadian canola cultivar Regent. Hybrids showed significant heterosis for seed yield, total dry matter, and apparent harvest index. Marnoo x Regent, and Karat x Regent F_1 seed yield exceeded Regent by 38 and 43% respectively (Sernyk and Stefansson, 1983; Sernyk, 1983). This research suggests that commercial exploitation hybrids with seed yield up to approximately 40% is possible if parents with suitable combining ability are selected. Grant and Beversdorf (1985) conducted a 6 x 6 diallel cross with summer rape and found high-parent heterosis for seed yield up to 72%, again confirming hybrid potential in rape. Similarly, Lefort-Buson and Dattee (1985a) reported positive heterosis when comparing two populations of winter rape for which most genetic variability was attributed to general combining ability (Lefort-Buson and Dattee, 1985b). Lefort-Buson *et al.* (1987c) studied hybrids from crosses within and between European and Asiatic lines of inbred rape and compared heterosis in relation to genetic distance. Hybrids between lines of

similar origin gave low heterosis while crosses between diverse genotypes, European x Asiatic crosses, gave higher levels of heterosis. Crosses within each group predominantly expressed general combining ability which was interpreted to be caused by additive genetic effects while both general and specific combining ability was significant in crosses between lines of different geographic origin. The specific combining ability was suggested to be due to 'good genetic complementarity between European and Asiatic material' as a consequence of the interaction among new dominant alleles and epistatic effects at various loci (Lefort-Buson *et al.*, 1987c). The suggestion that average F_1 heterosis tends to increase with genetic distance is supported by several authors (Shinga, 1976; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson *et al.*, 1987abc).

Heterosis for seed yield in intercultivar oilseed rape hybrids of up to 70% higher than mid-parent have been demonstrated (Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985). Hybrid vigour of F_1 hybrids derived from intercultivar crosses represents an average heterosis based on the individuals selected from each cultivar (Hallauer and Miranda, 1981) so heterosis is not generally repeatable unless the parent cultivars are homozygous. Since rape cultivars are genetically heterogeneous (Brandle and McVetty, 1989b), selection of inbred lines that maximize heterosis when crossed are most desirable (Allard, 1960). Brandle and McVetty (1989a) compared hybrids derived from crosses between inbred lines with hybrids obtained from crosses between cultivars and found yield of the former significantly higher than the latter in some crosses between inbed lines, attributable primarily to general combining ability. This research supports using crosses between inbred lines, rather than intercultivar crosses, to maximize seed yield of F_1 hybrid rape.

2.6 Synthetics

Production of synthetics offer controlled utilization of an appreciable amount of heterosis and is an alternative to the higher yielding, but more costly production of single-cross hybrids (Allard, 1964). Production of synthetic varieties in rape, the crossing *inter se* of selected genotypes, has been suggested provided lines exhibit good combining ability and sufficient cross pollination (Schuster and Michael, 1976; Schuster, 1982; and Schuster *et al.*, 1985). Rape synthetic varieties have been reported to yield up to 10% more than their components at natural outcrossing rates (Schuster, 1982), but natural outcrossing rates utilizing partial allogamy have limited ability to exploit the full potential of hybrid rape. Synthetics based on a pollen control mechanism, such as sporophytic incompatibility, would have higher rates of outcrossing and better ability to exploit heterosis. Banks (1988) simulated the performance of summer rape synthetics based on sporophytic incompatibility by using hand pollination. Heterosis for seed yield between 2-, 3-, 4-, and 5-parent synthetic-1s was not significantly different and averaged 27% higher than the mean of the cultivars used as parents. Synthetic-2 and synthetic-3 generations produced by random mating exhibited the yield decline expected from inbreeding depression of a cross-pollinating species (Banks, 1988). By using sporophytic control to produce synthetics, it may be possible to commercially produce F_1 hybrid rape using inbred lines with superior combining ability.

2.7 Pollination

Pollination of the predominantly autogamous, self-fertile rape by wind plays a significant role in normal seed establishment (Olsson, 1955; Williams, 1978; Mesquida and Renard, 1982) but some evidence suggests that the honey bee (*Apis mellifera* L.) is the main pollinating agent in rape (Mesquida and Renard,

1979). There is disagreement as to the requirement of entomophilous pollination for good yield in rape that autopollinates well (see: Mesquida *et al.*, 1988). Even though rape is self-fertile, some external agent appears necessary to transfer enough pollen from anthers to stigma to ensure good seed set (Williams, 1985) possibly because oilseed rape is typified by sticky pollen with typical entomophilic pollen characters (Eisikowitch, 1981).

Insect and wind pollination vectors are essential for adequate cross-pollination of many allogamous species such as turnip rape (Williams, 1978; Holm *et al.*, 1985). F_1 hybrids developed from allogamous rape would hence require manipulation of field design, and insect activity to ensure good cross-pollination between pollen and seed parents. Planting design is of paramount importance for wind-effected pollination since direction of wind during pollination and seed to pollinator parent ratio influences the extent of outcrossing (Banga and Labana, 1983). Alternatively, selective elimination of the pollinator from mixed plants with techniques such as selective herbicide application (Barsby *et al.*, 1987) would allow harvest of the desired F_1 hybrids.

2.8 Pollen control mechanisms

2.8.1 Gametocides

No effective gametocides for commercial *Brassica* hybrid production are currently available. Van der Meer and Van Dam (1979) report that multiple applications of gibberellic acid prevented pollen shed in cabbages (*B. oleracea*) at early stages of flowering but not at later stages. Banga and Labana (1983, 1984) showed that the ethylene-generating synthetic compound 2-chloroethyl phosphomic acid (ethrel, ethephon) was effective in pollen retardation of *B. juncea*, with treated varieties up to 90% male sterile (Banga and Labana, 1984). Guan and Wang (1987) also reported 3 unidentified

compounds (MG_1, MG_2, and MG_3) which induced 60-80% male sterility, and a review by Kaul (1988) mentions that zinc methyl arsenate induces some male sterility in *B. napus*. Gametocides tested to date in *Brassicas* are not of commercial value for hybrid production, but induced anther retardation can be useful in breeding procedures requiring increased rates of outcrossing such as recurrent selection programmes.

2.8.2 Genetic Male Sterility

Male sterility in plants is the complete or partial failure to produce functional anthers, pollen or male gametes. Genetic male sterility is controlled either exclusively by the nuclear genes or by the complementary action of nuclear and cytoplasmic genes. The former is termed genic male sterilty and the latter gene-cytoplasmic male sterility, both of which are extensively reviewed by Kaul (1988).

2.8.3 Genic male sterility

Genic male sterility (GMS) has been identified in *B. napus* (Takagi, 1970; Mathias, 1985a; Li *et al.*, 1988) but has not been used in commercial hybrid seed production. The use of GMS in hybrid seed production has been limited since maintenance of pure GMS lines is difficult, roguing male fertile plants is labour intensive, yield is limited when cross-pollination is inadequate, and GMS flowers are less attractive to pollen-collecting insects (Kaul, 1988). However, research at the Shanghai Academy of Science in China indicates commercial hybrid rape production may be feasible (Li *et al.*, 1988 and references therein). Li *et al.* (1988) discovered GMS in a rape cultivar determined by two loci, designated MS_1 and MS_2. Plants homozygous or heterozygous for the dominant MS_1 allele (MS_1MS_1 or MS_1ms_1), and homozygous for the

recessive allele at the second locus (ms_2ms_2) are sterile. Plants homozygous or heterozygous for the dominant MS_2 allele are fertile regardless of the alleles present at the MS_1 locus indicating dominant epistasis of the MS_2 locus over the MS_1 locus. Plants homozygous for recessive alleles at both loci are also fertile. Hybrid seed is produced by crossing $MS_1MS_1ms_2ms_2$ (male sterile) x $ms_1ms_1ms_2ms_2$ (fertile) to give $MS_1ms_1ms_2ms_2$ (male sterile) progeny. Hybrid F_1 rape is obtained by crossing $MS_1ms_1ms_2ms_2$ with _ _ MS_2MS_2 lines producing a generation of fertile plants. Preliminary seed yield trials using this diallelic Chinese GMS system have shown F_1 hybrids yielding more than conventional cultivars by more than 30% (Li *et al.*, 1988).

2.8.4 Cytoplasmic (gene-cytoplasmic) male sterility

Utilization of cytoplasmic male sterility (CMS) as a pollen control mechanism to exploit heterosis for seed yield in hybrid rape has received considerable attention in the last two decades, yet successful commercial application requires formidable research. In addition to CMS rape (A-lines), maintainers (B-lines) and restorers (R-lines) with suitable combining ability and ability to restore F_1 fertility are necessary. Seed set of A-lines is influenced by factors such as: high level of fertility; ratio of A-lines to pollinating lines; distance of A-lines to B- or R-lines and planting design; synchronization of male and female flowering time; morphology of lines; attractiveness of flowers (such as floral physiology and quality of nectar production) to insect pollinators; and environmental parameters such as temperature and wind (reviewed by Frauen, 1987; Röbbelen, 1987). Despite these limitations several CMS systems have been considered for F_1 hybrid rape production (reviewed by Shiga, 1980; Kaul 1988).

2.8.5 Naturally occurring cytoplasmic male sterility in *B. napus*

Shiga and Baba (1971, 1973) reported CMS *B. napus* derived from intraspecific crosses between Hokuriku and Chisaya-natane. Thompson (1972) independently reported that crosses between Bronowski summer rape (male parent) with either summer or winter rape resulted in F_2 progeny plants segregating for male sterility. Flowers of the male sterile plants used by both Shiga and Baba (1971, 1973) and Thompson (1972) were characterized by short filaments, narrow petals, and small anthers with few or no pollen. Shiga's male sterility was considered to be the same as Thompson's (Shiga *et al.*, 1983). Although selection of good maintainers are possible since most rape cultivars are able to restore fertility, male sterility in this system is unstable at day/night temperature regimes at or exceeding 26/20 °C (Fan and Stefansson, 1986), thus limiting commercial potential.

2.8.6 Polima system.

Male sterility has been reported in the cultivar Polima (Dong, 1981). Fan and Stefansson (1986) found this CMS system expresses partially male fertility at day/night temperature regimes of 30/24 °C. Despite this limitation, all *B. napus* cultivars tested by Fan *et al.* (1986) were partial or complete maintainers of the Polima male-sterile lines. Fan *et al.* (1986) also found restorers from the *B. juncea* cultivar ZEM and additional restorers from winter rape have been reported by Fang and McVetty (1987, 1989). Tai and McVetty (1988) demonstrated complete restoration of Polima CMS *B. napus* following crosses to ZEM. An additional pair of chromosomes was observed in fully restored plants, presumably derived from *B. juncea*. Since a complete CMS system has been identified, with A-, B- and R-lines, the Polima cytoplasm is considered to have the most immediate potential for CMS hybrid rape production. Indeed, the

CMS summer rape hybrid 'Hyola 40' is the first Canadian registered hybrid summer rape (registered in 1989) and was developed using the Polima cytoplasm system. It should be noted, however, that the performance of male fertility restored hybrids varies depending on the male sterility inducing cytoplasm. McVetty *et al.* (1990) reported that F_1 hybrids with the polima cytoplasm performed significantly lower than F_1 hybrids in the *B. napus* (*nap*) cytoplasms with respect to seed yield, total dry matter, harvest index, percent oil, total oil yield, and total protein yield.

2.8.7 Cytoplasmic male sterile *B. napus* in radish cytoplasm

Ogura (1968) discovered a male-sterility-inducing cytoplasm in Japanese radish (*Raphanus sativus* L.) where male sterility is controlled by homozygous recessive nuclear genes (msms) and a sterility-inducing cytoplasm (S). All Japanese varieties were homozygous recessive, msms, carrying no restorer gene. Bannerot (1974) transferred the cabbage (*B. oleracea*) nucleus into the CMS radish and later developed CMS *B. napus* by substitution backcrosses with the CMS *B. oleracea* (Bannerot, 1977). Initial male sterile plants were very stable and easily maintained but unattractive for insect forage due to reduced number of nectaries and nectar production plus chlorosis at low temperatures (Rousselle, 1982). Chlorophylous non-deficient plants were later developed by protoplast fusion (Pelletier *et al.*, 1983) making bee forage more attractive (Renard and Mesquida, 1987ab) and subsequent hybrid production more plausible. Restored plants from crosses with available restorer lines derived from *Raphanus* and *Raphanobrassica* have reduced female fertility (Heyn, 1988) to an extent exceeding the benefits of heterosis (Pellan-Delourme *et al.*, 1987). Commercial development of hybrid rape using this system awaits selection of superior restorer genes.

2.8.8 *Diplotaxis muralis* system

Hinata and Konno (1979) developed CMS *B. campestris* by intergeneric hybridization with *Dipotaxis muralis* DC. followed by backcrossing. Pellan-Delourme and Renard (1987) reported that most *B. napus* cultivars carried restorer genes for the *D. muralis* cytoplasm, but only two rape cultivars, Mangun and Hinchu, of 147 tested were able to maintain male-sterility. Although numerous restorer genes are available for the *D. muralis* cytoplasm, backcrosses with Mangun and Hinchu resulted in low seed set thus limiting the commercial potential of this system.

Several other CMS systems describe interspecific crosses with male-sterility-inducing cytoplasms of *B. juncea* (Mathias, 1985b), *B. oleracea* (Rousselle *et al.*, 1983), and *B. nigra* (Pearson, 1972) but these systems are incomplete lacking either restorers, maintainers, or sufficient seed set to warrant commercial hybrid production.

2.8.9 Self-incompatibility

Self-incompatibility is widespread in angiosperms and establishes panmixis by preventing self-fertilization from occurring in allogamous plants. Although there are many manifestations of self-incompatibility, the principle effect is to ensure preferential cross-fertilization. The literature is not clear as to whether the term "self-incompatibility" should be restricted to pre-fertilization processes or extended to describe all events which prevent fertile hermaphrodites from setting seeds upon selfing (Nettancourt, 1977). A generally accepted definition for self-incompatibility is "the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination" (Lundqvist, 1964). The genetics, physiology, and evolutionary importance of self-incompatibility has been reviewed by numerous authors (Lewis, 1949a, 1960, 1976, 1979; Bateman,

1952; Pandey, 1960, 1979; Crowe, 1964; Linskens, 1965; Lundqvist, 1965; Arasu, 1968; Townsend, 1971; Nettancourt, 1972, 1977, 1984; Dickinson and Lewis, 1973a,b; Heslop-Harrison *et al.*, 1974,1975ab; Nettancourt and Devreau, 1977; Clarke and Knox, 1978; Heslop-Harrison 1978, 1983; Harris *et al.*, 1984; Knox *et al.*, 1984).

Discrimination of self- from cross-compatibility is rarely brought about by contact between male and female gametophytes. Evolution of siphonogamy in the angiosperms has permitted the direct interaction between the male gametophyte and the female sporophyte before fertilization. Since gamete transfer necessitates a pollen tube passing through the female sporophyte to reach the embryo sac, male gametes are open to control by the female prior to syngamy. Regulation of pollen tube germination on the stigmatic papillae, tube growth through the style, and fertilization in the ovary may be controlled by recognition and regulation of the genome of the male gametophyte (gametophytic incompatibility). Alternatively, control may be conveyed by the pollen as a product of the male-acting sporophyte (sporophytic incompatibility) (Heslop-Harrison, 1983).

In several families of flowering plants, incompatibility is attributed to a single multi-allelic locus, the S-gene (Nettancourt, 1977). If the alleles expressed by both pollen and pistil are identical, an incompatibility reaction prevents syngamy. Self-incompatibility systems are classified as being heteromorphic or homomorphic. Heteromorphic families have polymorphic flowers with morphologically distinct differences in the relative positions of their stigma(ta) and anthers (Crowe, 1964) and all exhibit sporophytic incompatibility. Homomorphic families such as Brassicaceae show no floral polymorphism and have been further classified into gametophytic (GI) and sporophytic (SI) incompatibility on the basis of the time and site of S gene action.

2.8.10 Gametophytic incompatibility

In gametophytic systems, the incompatibility phenotype of a pollen grain is controlled by its own haploid genotype (Nettancourt, 1977). Growth of the pollen tube is arrested in the style and syngamy prevented if the pollen S allele is identical to the S allele of the recipient plant. Control is usually mediated through a single gene locus (S), but more complex systems have been described such as the two gene system (S and Z) in Gramineae, three S-locus system in *Ranunculus acris*, and four genes in *Beta vulgaris* (Harris *et al.*, 1984).

Until recently, the Brassicaceae was characterized by the sporophytic control system. However, Zuberi *et al.* (1988) and Lewis *et al.* (1988) have reported that *Brassica campestris* and *Raphanus sativus* respectively are typified by a linked GI-SI incompatibility system implying that the system may be common to all of the Brassicaceae. This work resolves many of the unexplained and unexpected anomalies reported in past publications, frequently in the nature of syngamy where incompatibility is expected. Extensive testing was necessary to confirm the weak influence of GI in the self-incompatible response of *B. campestris* and *R. sativus*. Screening for GI in *Brassicas*, or introgression of GI into appropriate *Brassica* species may yield lines suitable for future hybrid analysis, but to date, no literature has suggested that GI would be useful as an effective pollen control mechanism in this genus. For this reason, further discussion will be restricted to sporophytic incompatibility in *Brassica*.

2.8.11 Sporophytic incompatibility

The initial discovery of the sporophytic incompatibility system (Gerstel, 1950; Hughes and Babcock, 1950; Bateman, 1954) has lead to established

rules of mating patterns and segregation between pollen and stigma. Incompatibility in sporophytic systems is determined by the phenotype of the pollen rather than its haploid genotype. Determination of the pollen phenotype is accomplished through transfer of exine-borne fractions derived from the anther tapetum of the sporopyhte (Heslop-Harrison *et al.*, 1975b). The behaviour of the pollen in sporophytic systems governed by a single locus, as in Brassicaceae, and depends on the interactions of the two alleles borne by the diploid parent sporophyte from which the pollen was derived. Numerous alleles have been reported in *Brassicas*. Ockendon (1982) reported 31 S-alleles in a survey of 197 cabbage plants (*B. oleracea*) representing 11 diverse cultivars. The genetics governing sporophytic incompatibility is very complex. From the multiple alleles in a given population, the two different alleles in a heterozygote may be codominant, one allele may be dominant, or individual alleles may express different dominance relationships in pollen and stigma.

Unlike the gametophytic system where pollen tubes enter the pistil, SI pollen grains fail to germinate or, if germination is successful, produce pollen tubes that coil at the surface of the papillar cells and fail to penetrate the stigma or are arrested shortly after penetration. On the basis of physiological, genetic, and biochemical analysis, several hypotheses have attempted to explain this recognition event (see review by Nettancourt, 1977 and references therein). Although the SI system in *Brassica* is a well documented phenomenon of biological recognition, the precise mechanism is not well understood (see Roberts *et al.*, 1979; Roberts *et al.*, 1980; and Sarker *et al.*, 1988). Recent evidence suggests that an interaction between enzymes of the stigma surface and the superficial layer of the pollen grain coating creates continuity between the papillar wall and the grain protoplast thus regulating pollen hydration and potentially regulating compatibility (Sarker*et al.*, 1988). Numerous reports

suggest that S-locus-specific proteins (SLSG), S-glycoproteins, are the direct translational products of the S-gene (Nasrallah and Wallace, 1967; Nishio and Hinata, 1977ab, 1978, 1979, 1982; Okazaki and Hinata, 1984) in the stigma and are involved with the expression of self-incompatibility (Okazaki and Hinata, 1984; Hinata *et al.*, 1982; Nasrallah *et al.*, 1972,1985ab, 1987ab; Nasrallah and Nasrallah, 1986abc, 1988; Hinata and Nishio, 1978).

The stigmas of SI *Brassica* are initially self-compatible but become self-incompatible approximately 1 day prior to anthesis (Shivanna *et al.*, 1978; Roberts *et al.*, 1979). The shift to self-incompatibility has been associated with the accumulation of SLSGs (Hinata and Nishio, 1978; Nasrallah et al., 1985ab, 1987b, 1988 and references therein). The level of S-gene transcripts and their protein products have been reported to increase in stigmas during development of SI flowers, reaching a threshold point 1 day prior to anthesis which switches the pollen-stigma response from self-compatible to self-incompatible (Nasrallah *et al.*, 1985ab, 1987b) with a short lived transitional state (Nasrallah *et al.*, 1985a). Self-compatibility correlates well with developmental regulation of SLSG concentrations in the pistil since low levels of SLSGs have been detected in young buds by incorporation of labelled amino acids in vivo (Nasrallah *et al.*, 1985a), by immunochemical techniques (Nasrallah, 1974), and sensitive protein staining (Nasrallah and Nasrallah, 1984) while the shift to self-incompatibility in more mature buds is correlated with increased synthesis of SLSGs (for a full discussion, see Nasrallah and Nasrallah, 1986c).

The sporophytic system is functional in polyploids, whereas the gametophytic self-incompatibility system breaks down in polyploids (reviewed by Nettancourt, 1977) due to interaction between alleles in the pollen. Of considerable evolutionary interest is the fact that the diploid species of *B. oleracea*, *B. campestris* and *B. nigra* are self-incompatible while their

allotetraploid derivatives, *B. napus*, *B. juncea* and *B. carinata* are all self-compatible (Bateman, 1955). There is no inherent reason why derived allotetraploids should not be self-incompatible. Baker (1955; cited by Nettancourt, 1977) suggests that colonization by single seeds may have contributed to the formation of several self-compatible (SC) species. Indeed, sporophytic compatibility of allotetraploid species may have been a derived condition from ancestral SI species necessary for primary colonization

Howard (1942) reported that autotetraploids were self-incompatible, suggesting polyploidy was not the cause for SC in the amphidiploid *Brassicas*. Mizushima (1950) found that many artificially produced allotetraploids in the *Brassicas* are self-sterile but fertile when outcrossed with sibs and related species. Olsson (1960a) reported that newly synthesized *B. napus* plants were often completely self-fertile while others were self-sterile yet autopolyploids of the parental species remained self-sterile (Olsson, 1953). *B. napus*, possesses two S-loci, one from each of the progenitor diploids. Hodgin (1986) reported that S-alleles from both *B. campestris* and *B. oleracea* were expressed in rape synthesized from parent plants homozygous for different alleles at their respective loci. In SC *B. napus*, neither of the S-alleles are active. But introgression of SI from *B. campestris* results in activity of *B. campestris* S-alleles in *B. napus* (MacKay, 1977). This suggests that SC rape is due to some type of genetic disturbance and not to deletions of the S-loci. The change from self-incompatibility to self-compatibility may be due to genes unrelated to S-loci (Hinata *et al.*, 1983; Nasrallah, 1974; Thompson and Taylor, 1971; Moine, 1985; Hinata and Okazaki, 1986) or to changes or interactions of the S alleles (Bateman, 1954; Thompson and Taylor, 1966b; Zuberi *et al.*, 1981). For example, Bateman (1954) assumed an Sf gene caused SC while Murakami

(1965) assumed F-f alleles at a locus different from S was responsible for sporophytic compatibility.

In any case, the point of practical interest here is that SI rape can be developed, although the genetics may be complicated. Complications arise from modifier genes inhibiting S-allele expression, epistatic interactions between S loci, dominance ($S_1 < S_2$) and codominance ($S_1 = S_2$) relationships, mutual weakening (no action by either S allele), intermediate gradations (0-100% activity by each S allele), and different genetic and physiological environments. From a breeders perspective, these factors will complicate selection of SI lines due to partial self-compatibility and aberrant segregations ratios when backcrossing to *B. napus*. Sporophytic incompatibility is normally a dominant trait, (Mackay, 1976, 1977) although recessive SI has been reported (Olsson, 1960a; Thompson, 1979, 1983; Thompson *et al.*, 1983). Dominance of SI is an obvious advantage in selection of SI rape lines permitting easy selection by counting selfed seed set or by using fluorescent staining of pollen tubes (Martin, 1959).

To successfully exploit heterosis in rape crosses using sporophytic incompatibility as a pollen control mechanism SI lines must be established. Naturally occurring SI plants of *B. napus* have been found in oilseed rape (Olsson, 1960a), and in swedes (Gowers, 1974, 1981). Successful synthesis of SI *B. napus* through interspecific hybridization of *B. campestris* and *B. oleracea* and related crosses such as *B. campestris* x *B. alboglabra* (a form of *B. oleracea*; see Prakash and Hinata, 1980) has been accomplished by Olsson (1960a), Chen *et al.* (1988b), and several other researchers (reviewed by Chen and Heneen, 1989b). Sporophytic incompatible rape lines have also been successfully developed by introgressing alleles from *B. campestris* (MacKay, 1977; Banks, 1988) and from SI Swedes (Banks, 1988). It is possible to

introgress SI into rape by crossing with *B. oleracea*, or by intergeneric crosses. However, these crosses are more difficult than using *B. campestris* (Olsson and Ellerström, 1980; Namai *et al*, 1980). Mutation screening for SI rape is impractical (for discussion see Nettancourt, 1977) and SI transformation of rape has not been reported, although the latter may soon be feasible in light of recent isolation of glycoprotein clones, involved in the self-incompatibility response, from *B. oleracea* (Nasrallah *et al*, 1985b,1987ab, 1988; Nasrallah and Nasrallah, 1989).

Production of hybrid cultivars of vegetable *Brassicas* has been reported (Haruta, 1962; Hinata and Nishio, 1980; Tsugimoto and Minata, 1981; Dickson and Wallace, 1986). The majority of commercial *Brassica* and *Raphanus* vegetables in Japan are produced by single or double crosses (Hinata and Nishio, 1980; but three-way crosses are also used (Tsujimoto and Minato, 1981; Figure 2.2).

Commercial production of F_1 rape using single crosses would not be possible unless cost effective methods of overcoming SI could be established to maintain isogenic lines. Indeed, a major drawback of using SI is the difficulty of producing commercial quantities of selfed seed from SI parent lines (Downey and Rakow, 1987). Some of the applications known to overcome or inhibit SI include: bud and delayed pollination (Shivanna *et al.*, 1978); stigma mutilation (Roggen and van Dijk, 1972) and stigma removal (Gowers, 1986); organic solvents (Roggen, 1979ab); phytohormones (Matsubara, 1985); proteins, and amino acids (Matsubara, 1985); flavanoids (Prabha *et al.*, 1981); electric aided pollination (Roggen, 1982); thermally aided pollination (Goni and Hinata, 1971); use of mentor pollen (Pandey, 1978, 1979; Roggen, 1975); high relative humidity (Ockendon, 1972); salt (NaCl) solutions (Guohua and Rui, 1986; Monteiro *et al.*, 1988ab); sugars (Baja and Shivanna, 1986) and carbon dioxide

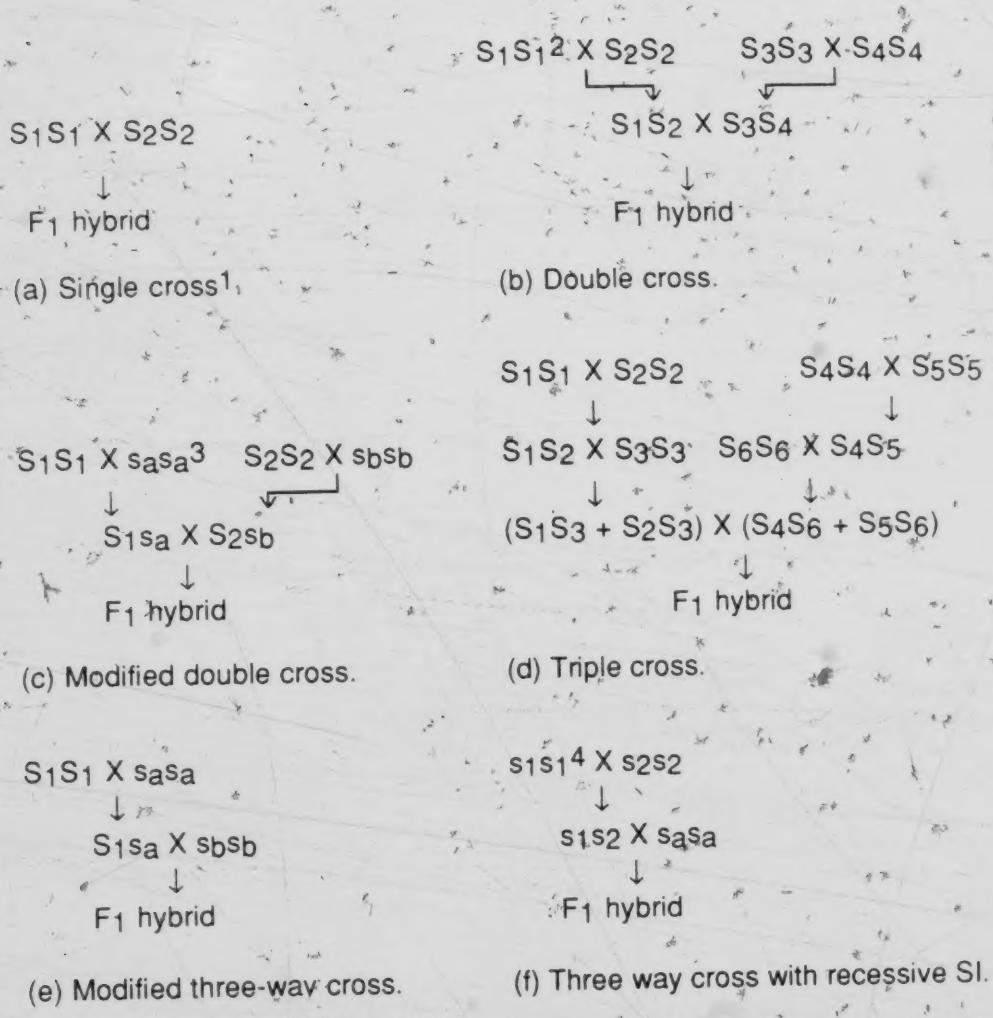


Figure 2.2 Breeding schemes for production of F_1 hybrid rape using dominant and recessive sporophytic incompatibility as the pollen control mechanism. (a) Single cross method using 2 inbred lines homozygous for different dominant SI alleles. (b) Double-cross method (Thompson, 1959) using 4 dominant SI lines. (c) Modified double-cross (Gowers, 1975) using 2 dominant SI lines and 2 SC (recessive) lines. (d) Triple-cross (Thompson, 1964) using 6 dominant SI lines. (e) Modified three-way cross (Gowers, 1975) using 1 dominant SI line and 2 (recessive) SC lines. (f) Three-way cross (Thompson, 1979) using 2 recessive SI lines and 1 (recessive) SC line.

1- Note that the F_1 from the single cross is self-sterile so would be of value in vegetable production, but of no value for seed production.
2 - S_1, S_2, ..S_6 are isogenic inbred SI lines with dominant S alleles
3 - S_a and S_b are SC isogenic inbred lines recessive to S_1, S_2, ..S_6.
4 - s_1 and s_2 are recessive SI alleles.

(Nakanishi *et al.*, 1969; Nakanishi and Hinata, 1973, 1975; Dhaliwal *et al.*, 1979; Dhaliwal and Malik, 1981; Malik and Dhaliwal, 1986). (For further discussion on overcoming SI, see Hinata and Nishio, 1980; see Matsubara, 1985, for additional references).

The usual method of overcoming SI in *Brassica* has been to use bud pollination or carbon dioxide (CO_2) application. Bud pollination is suitable for production of small amounts of selfed seed, but is time and labour intensive making this system impractical for commercial application of SI oilseed lines.

Using CO_2 can be a labour saving, practical breeding approach to overcoming self-incompatibility since insects, such as bees, can be used as pollination vectors. Nakanishi *et al.* (1969) and Nakanishi and Hinata (1973) found many pollen tubes were penetrating the stigmas of *B. oleracea* after exposing flowers to 3-5% CO_2 for approximately 6 hours after self-pollination. Overcoming incompatibility in this way is presumed to involve ionic balance disturbances in the papillae (Hinata and Nishio, 1980). Thompson (1979) has successfully used CO_2 in breeding recessive SI *B. napus* (Figure 2.2 f). Carbon dioxide may be a practical method for breaking self-incompatibility (Malik, 1981) on a commercial scale. Taylor (1982), supporting this claim, found that in marrow-stem kale (*Brassica oleracea* var. *acephala* and in Brussels sprouts (*Brassica oleracea* var. *gemmifera*), treating with CO_2 was an effective aid in seed production. Even with the ability to overcome SI however, three-way or double-cross hybrids may be better for commercial production of rape than single-crosses (Gowers, 1989), since the former can be mass produced by natural pollination.

Carbon dioxide is effective in overcoming SI, but requires special facilities for application. An inexpensive and easily applied technique for overcoming SI has recently been reported by Guohua and Rui (1986). They found that a 3%

NaCl solution sprayed 30 minutes to 1 hour after pollination of Chinese cabbage (*B. campestris* ssp. *perkinesis*) was able to overcome SI. Subsequent studies using *B. campestris* have confirmed these findings and have determined that the optimum time and rate of NaCl application to be 15 minutes before pollination using 15 g liter-1 NaCl solution respectively (Monteiro *et al*., 1988ab). This technique promises to be useful for overcoming SI on a commercial scale.

Self-incompatibility has been used to produce hybrid cruciferous vegetables since 1942 (Tsujimoto and Minata, 1981) and one field crop, marrow stem kale, commercially available under the name Maris Kestrel since 1965 (Thompson, 1967). The development of Maris Kestrel with its production problems and advantages are particularly salient, and are reviewed by Banks (1988). Thompson (1959) proposed that hybrid marrow-stem kale could be produced by a double cross (Figure 2.2 b) using 4 isogenic SI lines. To save labour and improve seed multiplication in early stages of hybrid development Thompson (1964) introduced the "triple-cross" method based on six isogenic SI lines crossed over 3 generations (Figure 2.2 d). Maris Kestrel followed this evolution, developed originally as a double-cross hybrid, and ending up a triple-cross hybrid to satisfy production requirements (see Thompson, 1967; Banks, 1988, and references therein).

Gowers (1975) suggested a modified double-cross for hybrid swede production (Figure 2.2 c) which he latter extended to rape (Gowers, 1979, 1980). The advantage of this crossing scheme is that only one SI pair of alleles is required for each single cross instead of two, and that the proportions of compatible pollinations in the final field crop are increased. Using the standard double cross, with four S-alleles at the same locus, 25% of the field crop would be cross-compatible. In the modified double cross, half the plants would be

cross-compatible with half the population, and 25% would be completely cross-compatible (Gowers, 1979). Since a high frequency of cross-incompatible plants will reduce seed production, it is pertinent to determine the acceptable level of incompatibility in the final crop while maintaining full seed set. Gowers (1980) reported that enough compatible crosses, using a standard double cross, were available for full seed set. Sufficient cross pollination for full seed set may be inhibited however by inclement weather (Thompson, 1983; Thompson *et al.*, 1983), which may prevent cross-fertilization by wind (Olsson, 1955; Williams, 1978, 1984; Mesquida and Renard, 1982) or insects (Mesquida and Renard, 1979; Holm *et al.*, 1985; Williams, 1985).

Commercial application of a three-way cross using two recessive SI lines and one SC line (Figure 2.2 f) has been proposed (Thompson, 1979; Thompson *et al.*, 1983). The seed from this three-way cross is SC and would be expected to give full seed set. Inasmuch as the SC line in the second cross must be removed to maximize hybrid seed production, the SC line could be space planted and harvested separately from the F_1 x SC seed, or selectively removed by herbicide application to introgressed triazine resistant SI lines (Beversdorf *et al.*, 1980; Thompson *et al.*, 1983). Although this breeding scheme is sound, commercial hybrids have yet to be registered using this method. A significant limitation is that few genetically recessive SI lines have been reported (Thompson, 1979, 1983; Thompson *et al.*, 1983) which exhibit the strong SI, combining ability, and agronomic performance necessary to justify hybrid rape production.

Despite of the apparent obstacles to making SI a viable pollen control mechanism for rape hybrid production, the system has potential. Sporophytic incompatible F_1 seeds can be obtained from both parents, unlike male sterile systems. Isogenic CMS lines often exhibit deficiencies in A-line fertility and

floral morphology which has not been reported in isogenic SI lines. Hybrid root or vegetable crops with reduced F_1 fertility is inconsequential [e.g. note that F_1 seed from the single cross depicted in Figure 2.2 (a) would be of no value for seed production since the F_1 would be self-sterile], but full fertility of seed crops is essential if maximum yields are to be achieved. Complex crosses are also possible in the SI system, thereby reducing production costs. A major drawback in multiplying inbred SI lines (Van der Meer and Nieuwhof, 1968) has been largely overcome by CO_2 or NaCl application. While complex relationships of pollen-pistil S-allele interactions exist, so too do dominant alleles with simple inheritance (Johnson and Blyton-Conway, 1976; Banks, 1988).

3.0 Heterosis and combining ability estimates for five varieties and seven lines of summer oilseed rape (*Brassica napus* L.).

The objective of this research was to confirm reports that significant hybrid vigour for useful agronomic traits is present in F_1 summer rape.

3.1 Materials and Methods

Seven lines and 5 varieties (hereafter referred to as lines) of summer rape with Canadian and European origins (Table 3.1; see Appendix A for seed source) were selected on the basis of canola quality and divergent origins. The 12 lines were seeded in greenhouse conditions on June 28, 1985 and a complete diallel cross was made. Natural radiance in the greenhouse was supplemented with a 16-hour photoperiod of artificial light provided by GTE Sylvania Lucalux LU400 high pressure sodium lamps giving an average photosynthetic photon flux density of 200 ± 30 μmol m^{-2} s^{-1} at the soil surface measured using a Lambda LI-185 photometer fitted with a quantum sensor. The temperature for light and dark periods was 22 ± 2 °C and 15 ± 2 °C respectively. Single seeds of all lines were planted in 13 cm diameter pots containing a sphagnum peat-vermiculite-nutrient mix (Appendix B. Note: these greenhouse conditions remained constant for all tests reported on in this thesis unless otherwise indicated). Seed from the 132 F_1 hybrids was collected at maturity, hand threshed, and cleaned with a Dakota blower to remove chaff and shrivelled seed.

On February 27, 1986, parental and F_1 seed were sown in a 3 replication partially balanced lattice design in the greenhouse. To ensure uniform germination, 4 to 10 seeds were seeded in each pot, depending on seed availability. At the cotyledon stage of development seedlings were randomly thinned to 3 plants pot^{-1}. Each pot was considered as a plot and, with the

exception of days to flower, all agronomic parameters measured were based on the average performance of the plants within each pot. Days to flower were

Code	Line or variety	Origin	Institution of Origin
01	SV02231	Sweden	Svalof AB
02	Global	Sweden	Svalof AB
03	Hanna	Sweden	Weibull AB
04	Karat	Sweden	Svalof AB
05	ZN7-558	Canada	Agriculture Canada, Saskatoon
06	DI-820	Canada	Agriculture Canada, Saskatoon
07	Westar	Canada	Agriculture Canada, Saskatoon
08	77-1999B	Canada	University of Alberta
09	81-50864B	Canada	University of Alberta
10	Andor	Canada	University of Alberta
11	S76-3048	Canada	University of Manitoba
12	S76-3513	Canada	University of Manitoba

Table 3.1. *B. napus* lines and varieties used in a diallel cross.

recorded on first flowering of any plant in the pot. The agronomic parameters measured were: days to flower, days to mature, plant height, length of main raceme, number of siliques on the main raceme, yield, mg seed^{-1}, and percent seed oil content. The discussion and analysis which follow will be restricted to days to flower, yield, mg seed^{-1}, and percent oil.

At maturity, plots were hand cut, placed into paper bags, and dried on warm forced air drying racks for two weeks followed by manual threshing. Plot seed was screened, redried in an oven for 48 hours at 35 °C and total seed weight obtained. Sub-samples from each plot were cleaned using a Dakota blower and tested for thousand seed weight using a Model 701A Count-A-Pak electronic seed counter (Davis Tool and Engineering Inc., Montgomery, Illinois, 60538). One thousand (± 20) seeds were counted for each plot and weighed (± 0.001 g) for the mg seed^{-1} variable. Samples were then tested for oil content (± 0.1%) by nuclear magnetic resonance (NMR) using a Newport Analyser MK III A

(Oxford Instruments Group, England, MK14-5AW) calibrated with samples from which percent oil had previously been determined by the Swedish tube method.

Mean values for all crosses, general and specific combining ability effects, and mean specific combining ability effects were analyzed using 'Diallel Cross program S019' written by the Statistical Research Section, Engineering and Statistical Research Center, Agriculture Canada. General combining ability (g.c.a.) refers to the average performance of a line in hybrid combinations; specific combining ability (s.c.a.) designates cases which do relatively better or worse than would be expected on the basis of the average performance of lines involved (Sprague and Tatum, 1942); and reciprocal combining ability (r.c.a.) designates those cases in which the direction of the cross determines the performance of the lines involved.

The S019 diallel cross analysis was based on Griffing (1956) with modifications to standard errors as suggested by Thompson (1975). Since missing values are not estimated in the S019 program and introduce imbalance in the design (degrees of freedom and sum of squares are incorrectly calculated), missing values were replaced with estimated values generated from a lattice macro program using Genstat version 4.04B (Rothamsted Experimental Station, England). This resulted in increased confidence levels, but since there were few missing values, the effects were negligible.

Griffing's (1956) Method 1 Model II, restricted to randomized-block analysis, was used to analyze the diallel cross. The model is:

$$x_{ijkl} = u + v_{ij} + b_k + (bv)_{ijk} + e_{ijkl}$$

where u is the population mean, v_{ij} is the effect for the ijth genotype, b_k is the kth block effect, $(bv)_{ijk}$ is the interaction between the ijth genotype and the kth

block, and e_{ijkl} is the random variation associated with the ijklth individual. The variety effects for g.c.a. and s.c.a. is:

$$v_{ij} = g_i + g_j + s_{ij} + v_{ij}$$

where g_i is the g.c.a. effect of the ith parent, s_{ij} is the s.c.a. effect for the cross between ith and jth parents, and r_{ij} is the reciprocal effect involving the ith and jth parents. The model used for combining ability analysis is:

$$x_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{b}\sum_k b_k + \frac{1}{b}\sum_k (bv)_{ijk} + \frac{1}{bc}\sum_k\sum_l e_{ijkl},$$

where all effects are considered to be random variables except u (Griffing, 1956).

3.2 Results and Discussion

Preliminary analysis of variance using lattice Genstat (not shown) and subsequent randomized block analysis using S019 (Table 3.2) both showed significant differences for all agronomic traits measured ($P < .001$). Since genotypic differences among F_1 and parental plants was apparent, general, specific and reciprocal combining ability was tested and also found to be highly significant for all traits (Table 3.3). Tabular summaries for mean values of crosses, g.c.a, s.c.a., r.c.a., and mean specific combining ability effects are presented for days to flower, yield, mg seed^{-1}, and percent oil respectively.

Flowering date

Mean days to flower ranged from 36 to 52 days after planting (Table 3.4). Many of the s.c.a. effects were earlier (negative values) flowering than the parents in the cross (Table 3.5) with Canadian X European (and European X Canadian) crosses showing the earliest mean flowering (Table 3.17). Reciprocal combining ability was variable (Table 3.6) although some lines were generally poor as female and as male parents such as line 09 and line 02 respectively. Five of 7 Canadian lines showed g.c.a. effects for early flowering while 2 of the 4 European lines showed early g.c.a. flowering effects (Table 3.16). Global (line 02) exhibited very late g.c.a. for flowering.

Yield (g plot^{-1})

High levels of heterosis for F_1 yield over mid-parent values were observed for several hybrids (Table 3.7). Some high performing crosses include 02 X 08, 10 X 03, 01 X 02, and 02 X 09 which yielded 171%, 109%, 91%, 49% better than mid-parents respectively. Even allowing for small greenhouse plot size which leads to fluctuations more extreme than would be observed in standard field plot evaluation, hybrid vigour is abundantly demonstrated for yield in several hybrids, as is evident in s.c.a. effects (Table 3.8). Twenty-two of the 32 Canadian (female) X European (male) hybrids exhibited yield gains compared to the reciprocal crosses (Table 3.9) which tends to support Grant and Beversdorf's (1985) findings that Canadian X European crosses have generally more productive seed yield than the reciprocal cross. Global displayed very strong g.c.a yield effects while DI-820 (line 06) showed strong negative g.c.a. effects (Table 3.16). The European X Canadian (and reciprocal) crosses showed generally higher yield than either the Canadian X Canadian or European X European crosses (Table 3.17). The superior performance from

hybrids resulting from parental crosses of diverse origin has been well documented in crops such as corn. Similar corroborating evidence for *B. napus* has been reported (see Shiga 1976; Sernyk and Stefansson, 1983; Lefort-Buson *et al.*, 1987abc; Brandle and McVetty, 1989a and references therein).

Mg seed^{-1}

Mean values for mg seed^{-1} is shown in Table 3.10. The superior s.c.a. crosses for positive heterosis were 05 X 01, 09 X 03, and 12 X 07 (Table 3.11). Reciprocal effects were variable with 19 of 32 Canadian (female) X European (male) crosses exhibiting positive heterosis (Table 3.12), although the Canadian X European (and reciprocal) crosses exhibited less positive hybrid vigour than the European X European or Canadian X Canadian crosses (Table 3.17). Line 07 was a superior female parent (Table 3.12) and displayed the best g.c.a. for mg seed^{-1} effects (Table 3.16).

Percent Oil

Mean values for percent oil (Table 3.13) indicate several hybrids have significant gains in oil over mid-parents. Superior s.c.a. is indicated for crosses 02 X 01, 05 X 04, and 10 X 04 (Table 3.14) and r.c.a. effects were variable (Table 3.15). Global showed superior g.c.a. (Table 3.16), and positive heterosis for oil gain favored Canadian X European (and reciprocal) crosses more than Canadian X Canadian and European X European crosses (Table 3.17).

Source of variation	d.f	Days to flower		Yield (g plot^{-1})		Mg seed^{-1}		Percent oil	
		SS	MS	SS	MS	SS	MS	SS	MS
Blocks	2	41.456	20.728*[2]	88.843	44.421ns	3.561	1.781***	29.694	14.847*
Crosses	143	4823.473	33.731***	22767.092	159.210***	125.867	0.880***	3212.176	22.463***
Blocks*Crosses	286[1]	2228.983	7.794	12817.791	44.818	49.672	0.174	1263.485	4.418
Total	431	7093.912		35673.726		179.100		4505.355	
Number of missing values		10		14		10		10	
Estimated grand mean		44.41		22.74		3.97		36.41	

Table 3.2 Analysis of variance for five varieties and seven cultivars of *B. napus*.

1 - Blocks*Crosses d.f. varies depending on the number of missing values.
2 - *, ***, and ns indicate significance at $P < 0.05$, $P < 0.001$, and not significant respectively.

Variate:	d.f	Combining Ability Analysis: Days to flower		Yield (g plot^{-1})		Mg seed^{-1}		Percent Oil		Expectation of mean squares
		SS	MS	SS	MS	SS	MS	SS	MS	
General comb. ability	11	2832.02	257.46***[1]	9118.33	828.94***	77.460	7.042***	1922.86	174.81***	$\sigma^2 + 5.500\,\sigma_s^2 + 72.000\,\sigma_g^2$
Specific comb. ability	66	923.67	14.00***	6069.84	91.97***	26.889	0.407***	669.92	10.15***	$\sigma^2 + 5.542\,\sigma_s^2$
Reciprocal effects	66	1067.78	16.178***	7578.93	114.83***	21.518	0.326***	619.39	9.38***	$\sigma^2 + 6.000\,\sigma_r^2$

Table 3.3 Combining ability analysis for days to flower, yield, mg seed^{-1}, and percent oil in five varieties and seven cultivars of *B. napus*.

1 - *** indicates significance at $P < 0.001$.

	Male											
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	43.7	47.0	44.0	44.7	39.0	41.3	40.0	40.0	43.7	42.3	43.3	42.7
02	47.3	52.0	45.7	50.2	50.3	46.7	50.0	49.2	51.3	52.0	49.7	49.0
03	41.0	47.3	45.3	46.3	42.0	42.0	41.7	41.0	42.0	44.0	43.3	45.3
04	47.3	49.3	47.7	50.3	45.7	43.3	45.3	39.7	44.3	41.0	45.3	45.3
05	42.7	46.7	42.3	48.3	41.3	41.7	43.3	42.0	46.3	42.0	42.3	44.3
06	41.0	44.3	44.7	51.4	39.7	39.7	40.3	42.0	41.0	42.0	46.0	42.3
07	42.0	48.7	45.3	46.0	42.7	39.7	44.0	40.0	42.7	37.0	46.0	47.0
08	39.7	44.7	40.0	46.3	46.3	43.3	42.7	42.0	39.7	42.0	46.0	44.3
09	39.7	46.7	43.3	42.3	43.7	40.3	41.0	41.3	42.0	37.7	46.9	45.3
10	42.0	44.7	45.7	43.7	47.3	40.3	42.0	36.0	47.9	41.7	46.0	41.7
11	46.3	49.7	45.7	46.3	48.0	49.3	44.7	48.7	45.3	44.0	46.3	47.7
12	42.0	51.0	45.0	51.3	47.7	41.6	48.7	47.3	45.3	43.7	47.7	46.0

Table 3.4 Mean day to flower values for crosses.

SED = 2.02
LSD = $t_{(.05),244}$ = 3.98

	Parent											
	01	02	03	04	05	06	07	08	09	10	11	12
Parent												
01	2.55											
02	0.25	-0.71										
03	0.15	-1.65	1.74									
04	1.31	-0.71	1.08	2.08								
05	-1.56	0.31	-1.46	1.04	-2.34							
06	0.16	-1.30	1.09	2.81	-1.62	-1.23						
07	-0.88	1.66	0.38	0.22	-0.16	-1.77	1.36					
08	-1.28	0.04	-1.85	-1.68	1.78	1.66	-0.54	0.89				
09	-0.09	1.44	-0.33	-2.00	1.96	-0.98	-0.69	-1.25	-0.41			
10	0.97	1.34	2.41	-2.42	2.19	0.08	-2.46	-2.19	0.93	0.39		
11	0.19	-0.77	-1.38	-2.38	-0.75	3.14	-0.07	2.70	0.83	0.28	-1.83	
12	-1.78	0.09	-0.18	0.65	0.61	-2.04	2.96	1.73	0.58	-1.53	0.03	1.11

Table 3.5 Specific combining ability effects (S(I,J)) for days to flower

SE(S(I,I)) = 2.559
SE(S(I,I)-S(J,J)) = 3.604
SE(S(I,J)) = 1.817
SE(S(I,I)-S(I,J)) = 3.323
SE(S(I,I)-S(J,K)) = 3.121
SE(S(I,J)-S(I,K)) = 2.673
SE(S(I,J)-S(K,L)) = 2.548

	Male											
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	0.00											
02	0.17	0.00										
03	-1.50	0.83	0.00									
04	1.33	-0.45	0.67	0.00								
05	1.83	-1.83	0.17	1.33	0.00							
06	0.17	-1.17	1.33	4.06	-1.00	0.00						
07	1.00	-0.67	1.83	0.33	-0.33	-0.33	0.00					
08	-0.17	-2.28	-0.50	3.33	2.17	0.67	1.33	0.00				
09	-2.00	-2.33	0.67	-1.00	-1.33	-0.33	-0.83	0.83	0.00			
10	-0.17	-3.67	0.82	1.35	2.67	-0.83	2.50	-3.00	5.11	0.00		
11	1.50	0.00	1.17	0.50	2.83	1.67	-0.67	1.33	-0.77	1.50	0.00	
12	-0.33	1.00	-0.17	3.00	1.67	-0.37	0.83	1.50	0.00	-0.33	0.00	0.00

Table 3.6 Reciprocal effects (R(I,J)) for days to flower.

SE(R(I,J)) = 1.974
SE(R(I,J)-R(K,L)) = 2.792

	Male											
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	22.9	48.6	35.7	20.1	17.1	13.4	16.5	6.9	15.3	25.1	12.2	13.6
02	29.8	28.1	29.5	31.9	36.5	24.0	25.8	44.3	40.4	31.9	26.5	23.4
03	15.9	29.9	20.0	23.2	19.9	15.5	21.6	10.2	10.9	25.7	24.3	21.1
04	31.9	29.7	21.9	18.9	28.0	20.3	17.1	17.7	27.2	23.9	23.2	22.8
05	27.5	30.2	21.6	41.1	21.7	16.8	20.8	21.7	37.7	24.0	22.6	16.9
06	12.3	24.8	28.2	17.7	19.0	11.3	16.9	19.0	18.9	17.3	19.0	19.1
07	17.8	30.4	25.4	31.2	20.4	15.1	17.3	20.5	15.8	19.3	24.0	23.0
08	22.5	30.3	22.7	30.4	27.2	23.0	28.7	23.8	27.6	26.0	27.7	20.6
09	13.7	37.4	26.3	13.9	17.5	12.5	17.4	18.5	26.0	16.8	12.2	22.1
10	15.0	40.1	38.5	34.7	31.7	10.7	21.5	10.2	19.9	16.8	24.8	20.2
11	22.8	26.7	23.2	26.4	26.5	16.5	22.1	16.8	22.3	15.7	20.7	22.7
12	25.5	31.8	23.7	21.5	18.7	14.3	17.2	22.9	20.0	22.8	16.8	26.3

Table 3.7 Mean yield (g plot^{-1}) values for crosses.

SED = 5.34
LSD = $t_{(.05),240}$ = 10.51

Parent	Parent 01	02	03	04	05	06	07	08	09	10	11	12
01	3.62											
02	9.23	-12.45										
03	4.40	-2.32	-3.42									
04	3.00	-2.81	-2.54	-7.82								
05	-0.44	-0.01	-4.03	8.15	-4.40							
06	-2.78	-1.88	4.15	-0.31	-1.15	-0.66						
07	-2.07	-1.75	2.16	1.25	-2.01	0.40	-1.82					
08	-6.17	5.78	-6.55	-0.56	0.12	3.80	3.77	1.37				
09	-5.28	8.51	-3.25	-2.93	4.41	-0.41	-3.10	1.68	5.73			
10	-1.12	4.19	8.87	4.45	3.26	-3.51	-0.69	-4.64	-3.28	-6.15		
11	-2.28	-3.82	1.87	1.32	1.38	1.66	3.35	0.87	-3.00	-1.37	0.44	
12	-0.11	-2.68	0.67	-1.19	-5.28	0.69	0.51	0.53	0.91	-0.00	-0.38	6.33

Table 3.8 Specific combining ability effects (S(I,J)) for yield (g plot^{-1}).

SE(S(I,I)) = 6.137
SE(S(I,I)-S(J,J)) = 8.643
SE(S(I,J)) = 4.357
SE(S(I,I)-S(I,J)) = 7.968
SE(S(I,I)-S(J,K)) = 7.485
SE(S(I,J)-S(I,K)) = 6.410
SE(S(I,J)-S(K,L)) = 6.111

Fernale	Male 01	02	03	04	05	06	07	08	09	10	11	12
01	0.00											
02	-9.40	0.00										
03	-9.99	0.20	0.00									
04	5.88	-1.09	-0.67	0.00								
05	5.20	-3.15	0.87	6.56	0.00							
06	-0.55	0.43	6.37	-1.28	-1.07	0.00						
07	0.61	2.28	1.90	7.03	-0.20	-0.90	0.00					
08	7.80	-6.97	6.24	6.35	2.75	2.02	4.08	0.00				
09	-0.82	-1.52	7.73	-6.63	-10.07	-3.17	0.80	-4.57	0.00			
10	-5.05	4.10	6.42	5.42	3.85	-3.30	1.12	-7.88	1.57	0.00		
11	5.27	0.07	-0.57	1.60	1.95	-1.25	-0.97	-5.47	5.05	5.27	0.00	
12	5.93	4.22	1.27	-0.65	0.92	-2.42	-2.88	1.17	-1.03	5.93	-2.92	0.00

Table 3.9 Reciprocal effects (R(I,J)) for yield (g plot^{-1}).

SE(R(I,J)) = 4.734
SE(R(I,J)-R(K,L)) = 6.695

	Male											
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	3.54	3.58	3.34	3.01	3.73	3.81	3.26	3.63	3.17	3.16	3.26	2.72
02	3.12	3.86	4.53	3.43	4.53	4.62	4.89	4.25	3.87	4.27	3.75	3.92
03	3.06	4.14	3.82	3.04	4.20	3.86	4.22	3.66	3.20	3.97	4.77	3.14
04	3.23	3.20	2.86	3.56	3.41	3.52	3.01	3.74	3.38	3.35	3.34	3.10
05	4.50	4.03	4.09	3.16	4.50	4.58	4.86	4.20	4.33	4.40	4.15	4.13
06	3.46	4.40	4.00	3.69	4.43	3.50	4.81	4.51	4.43	4.45	4.35	4.04
07	3.91	4.28	3.92	3.39	4.95	4.85	4.66	4.62	4.41	5.15	4.65	4.99
08	3.79	4.66	3.96	3.13	4.52	3.94	4.01	4.04	3.98	3.88	4.43	4.09
09	3.50	4.31	3.62	3.59	4.23	4.66	4.28	4.12	4.14	3.97	3.89	4.39
10	3.36	4.75	4.70	3.14	4.56	3.66	4.51	3.68	4.30	4.19	4.30	4.02
11	2.99	4.96	3.46	3.34	4.04	4.72	4.12	4.27	4.29	4.20	4.41	4.19
12	3.67	3.54	4.52	3.50	4.20	4.04	4.64	4.15	4.14	3.58	3.94	3.92

Table 3.10 Mean mg seed^{-1} values for crosses.

SED = 0.35
LSD = $t_{(.05),244}$ = 0.69

	Parent											
	01	02	03	04	05	06	07	08	09	10	11	12
Parent												
01	0.65											
02	-0.22	-0.40										
03	-0.09	0.36	0.13									
04	0.34	-0.14	-0.22	0.89								
05	0.40	-0.12	0.03	-0.32	-0.04							
06	0.02	0.21	-0.08	0.09	0.06	-0.84						
07	-0.25	0.07	-0.17	-0.53	0.24	0.26	-0.13					
08	0.20	0.26	-0.10	0.03	0.01	-0.02	-0.14	-0.10				
09	-0.14	-0.07	-0.46	0.12	-0.02	0.34	-0.07	-0.05	0.08			
10	-0.27	0.30	0.40	-0.18	0.12	-0.20	0.35	-0.37	0.02	0.02		
11	-0.43	0.12	0.16	-0.10	-0.29	0.25	-0.11	0.18	-0.05	0.06	0.20	
12	-0.21	-0.35	0.03	0.01	-0.06	-0.08	0.48	0.10	0.28	-0.24	0.01	0.02

Table 3.11 Specific combining ability effects (S(I,J)) for mg seed^{-1}.

SE(S(I,I)) = 0.382
SE(S(I,I)-S(J,J)) = 0.538
SE(S(I,J)) = 0.271
SE(S(I,I)-S(I,J)) = 0.496
SE(S(I,I)-S(J,K)) = 0.466
SE(S(I,J)-S(I,K)) = 0.399
SE(S(I,J)-S(K,L)) = 0.380

		Male										
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	0.00											
02	-0.23	0.00										
03	-0.14	-0.20	0.00									
04	0.11	-0.12	-0.09	0.00								
05	0.39	-0.25	-0.05	-0.12	0.00							
06	-0.18	-0.11	0.07	0.08	-0.07	0.00						
07	0.33	-0.30	-0.15	0.19	0.04	0.02	0.00					
08	0.08	0.20	0.15	-0.30	0.16	-0.29	-0.31	0.00				
09	0.17	0.22	0.21	0.11	-0.05	0.12	-0.07	0.07	0.00			
10	0.10	0.24	0.37	-0.11	-0.80	-0.40	-0.32	-0.10	0.17	0.00		
11	-0.14	0.61	-0.66	-0.02	-0.06	0.19	-0.27	-0.08	0.20	-0.14	0.00	
12	0.47	-0.19	0.69	0.20	0.04	-0.02	-0.18	0.03	-0.12	0.47	-0.13	0.00

Table 3.12 Reciprocal effects (R(I,J)) for mg seed^{-1}.

SE(R(I,J)) = 0.295
SE(R(I,J)-R(K,L)) = 0.417

		Male										
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	34.0	39.9	38.0	36.7	38.8	30.9	36.9	32.4	36.2	36.6	37.7	35.5
02	41.0	35.1	39.0	40.3	40.0	38.3	37.7	42.0	39.8	37.0	38.5	39.5
03	35.9	40.4	38.3	40.0	37.4	33.8	36.0	38.2	39.1	38.8	35.0	38.9
04	37.9	40.5	38.5	36.4	40.4	35.1	36.2	37.4	37.2	38.8	36.9	37.2
05	36.7	42.2	39.0	42.0	38.0	31.0	37.8	38.7	40.2	38.7	36.1	36.6
06	34.7	35.0	37.8	39.4	34.7	28.0	30.6	31.6	32.4	29.9	34.5	34.7
07	34.6	38.4	38.6	39.9	36.1	32.1	32.0	33.8	37.4	34.4	33.2	33.2
08	35.3	34.7	37.5	35.9	37.3	32.1	35.9	36.6	36.8	37.8	35.4	37.2
09	34.0	39.2	39.7	37.5	38.5	33.4	36.9	34.9	35.3	33.7	35.9	38.8
10	35.2	34.4	37.5	39.7	37.6	34.2	35.7	31.9	33.6	31.1	36.3	37.4
11	35.8	35.1	35.8	38.3	37.5	31.2	34.7	34.4	36.0	32.5	35.4	33.0
12	35.6	41.0	35.9	41.4	38.2	31.1	34.9	37.5	35.7	36.1	37.3	34.4

Table 3.13 Mean percent oil values for crosses.

SED = 1.74
LSD = $t_{(.05),244}$ = 3.43

	Parent											
	01	02	03	04	05	06	07	08	09	10	11	12
Parent												
01	-1.57											
02	2.35	-5.50										
03	-0.45	-0.20	-0.87									
04	-0.60	-0.00	-0.51	-3.88								
05	-0.11	1.05	-1.15	1.35	-1.46							
06	0.16	1.42	1.31	2.24	-1.79	-1.82						
07	0.83	0.58	0.54	0.74	0.03	-0.75	-2.34					
08	-1.63	0.37	0.51	-1.17	0.52	-0.74	-0.04	1.14				
09	-1.04	0.84	1.43	-1.13	1.25	-0.38	0.16	-2.05	-1.41			
10	0.89	-1.81	-1.35	1.92	1.19	-0.06	0.67	-0.06	-1.91	-3.33		
11	1.70	-0.79	-1.49	0.16	-0.27	0.69	-0.51	-0.07	0.31	-0.11	0.80	
12	-0.51	1.69	-0.48	0.89	-0.61	-0.27	-1.38	1.37	0.63	1.28	-0.43	-2.19

Table 3.14 Specific combining ability effects (S(I,J)) for percent oil.

SE(S(I,I)) = 1.927
SE(S(I,I)-S(J,J)) = 2.713
SE(S(I,J)) = 1.368
SE(S(I,I)-S(I,J)) = 2.502
SE(S(I,I)-S(J,K)) = 2.350
SE(S(I,J)-S(I,K)) = 2.0124
SE(S(I,J)-S(K,L)) = 1.919

	Male											
	01	02	03	04	05	06	07	08	09	10	11	12
Female												
01	0.00											
02	0.57	0.00										
03	-1.03	0.73	0.00									
04	0.63	0.12	-0.75	0.00								
05	-1.35	1.13	0.77	0.82	0.00							
06	1.90	-1.65	1.97	2.15	1.88	0.00						
07	-1.15	0.38	1.28	1.83	-0.88	0.75	0.00					
08	1.43	-3.65	-0.34	-0.77	-0.68	0.27	1.08	0.00				
09	-1.08	-0.28	0.33	0.17	-0.87	0.52	-0.27	-0.95	0.00			
10	-0.68	-1.33	-0.67	0.42	-0.55	2.18	0.62	-2.97	-0.05	0.00		
11	-0.97	-1.67	0.40	0.70	0.70	-1.67	0.72	-0.50	0.04	-0.97	0.00	
12	0.02	0.77	-0.15	2.12	0.82	-1.76	0.83	0.13	-1.55	0.02	2.17	0.00

Table 3.15 Reciprocal effects (R(I,J)) for percent oil.

SE(R(I,J)) = 1.486
SE(R(I,J)-R(K,L)) = 2.102

Parent	Days to flower	Yield (g plot-1)	Mg seed-1	Percent Oil
01	-1.648	-1.742	-0.541	-0.423
02	4.149	8.906	0.144	2.094
03	-0.411	0.345	-0.143	1.394
04	1.923	1.959	-0.652	1.914
05	-0.370	1.682	0.288	1.541
06	-1.758	-5.397	0.188	-3.313
07	-0.884	-1.807	0.406	-1.036
08	-1.652	-0.152	0.082	-0.492
09	-1.005	-1.265	0.042	0.166
10	-1.567	0.107	0.101	-0.993
11	1.875	-1.251	0.121	-0.922
12	1.349	-1.384	-0.035	0.070
SE1	0.546	1.308	0.081	0.411
SE2	0.806	1.933	0.120	0.607

Table 3.16 General combining ability effects.

1 - SE for any effect.
2 - SE for difference of any 2 effects.

Cross	No. obs.	Days to flower	Yield	Mg seed-1	Percent oil
European X European	10	0.61 (0.57)[1]	-1.11 (1.38)	0.13 (0.01)	-1.12 (0.43)
European X Canadian	32	-0.20 (0.32)	0.07 (0.77)	-0.04 (0.05)	0.33 (0.24)
Canadian X Canadian	36	0.03 (0.30)	-0.02 (0.73)	0.01 (0.04)	-0.38 (0.23)

Table 3.17 Mean specific combining ability effects for European X European; European X Canadian (plus Canadian X European); and Canadian XCanadian crosses.

1 - (SE).

Significant heterosis was observed for all agronomic traits studied. The intervarietal heterosis observed is consistent with earlier studies (Schuster and Michael, 1976; Shiga, 1976; Morice, 1979; Buson, 1980; Guan *et al.*, 1980; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson *et al.*, 1987bc; Brandle and McVetty, 1989ab). The generally higher levels of heterosis observed between parents of diverse origin also support previous studies (Shiga 1976; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson *et al*, 1987abc; Brandle and McVetty, 1989b). Although g.c.a., s.c.a., and r.c.a. were all found to be significant (Table 3.3), g.c.a. accounted for the largest percent sum of squares for all traits measured (58%, 40%, 62%, and 60% for days to flower, yield, mg seed^{-1}, and percent oil respectively) suggesting additive genetic effects predominantly influenced expression of the agronomic traits observed.

The various analysis indicate significant heterosis is possible in F_1 summer rape. The question of cost of hybrid seed production versus relative economic gain for the producer is beyond the scope of this research since no attempt has been made to correlate possible crossing techniques and seed multiplication with relative yield gain and increases necessary for economic viability of hybrid seed production (see Frauen, 1987 for such a discussion). However, on the basis of favourable combining ability estimates of several lines and cultivars, the pursuit of a pollen control mechanism to exploit heterosis is amply justified.

4.0 Outcrossing in summer oilseed rape (*Brassica napus* L.) under Peace River, Alberta conditions.

In this experiment, progeny of low erucic acid summer rape from the cultivar Westar and 3 other lines (derived from the experiment of Rakow and Woods, 1987) were isolated and increased in the greenhouse. The low erucic acid seed was then seeded in a field trial using a geometric planting similar to that of Rakow and Woods (1987) and Banks (1988). The experiment was designed to: a) determine the rate of interplant summer rape outcrossing at one site in the Peace region in 1988, and b) determine whether the observed rates were attributable to genetic or environmental effects.

4.1 Materials and Methods

Rakow and Woods (1987) used Westar and 4 low erucic acid breeding lines to establish rates of *B. napus* outcrossing under Saskatoon conditions from 1982 to 1984 by geometric planting. Each low erucic acid cultivar or line was seeded 1.5 m distant from the next nearest low erucic acid entry into a block of the high erucic acid cultivar Reston. The assumption was that the wide dispersion of low erucic acid plants would reduce intracultivar/line pollination to a non-significant level. G. Rakow (Research Station, Agriculture Canada, Saskatchewan) kindly forwarded bulk seed harvested from 16 single plants harvested from this experiment. Single plants included Westar (A) and the low erucic acid lines DI 820 (B), 77-4397E (C), and WW48-29-80 (E). The interplant outcrossing rate from each of the 16 plants had previously been determined (Table 4.1) by Rakow and Woods by comparing the ratio of erucic acid containing seeds (cross-fertilized ovules giving rise to F_1 seed; e.g. Westar x

Reston) to low erucic seeds (self-pollinated ovules giving S_1 seed; e.g. self-pollination of Westar).

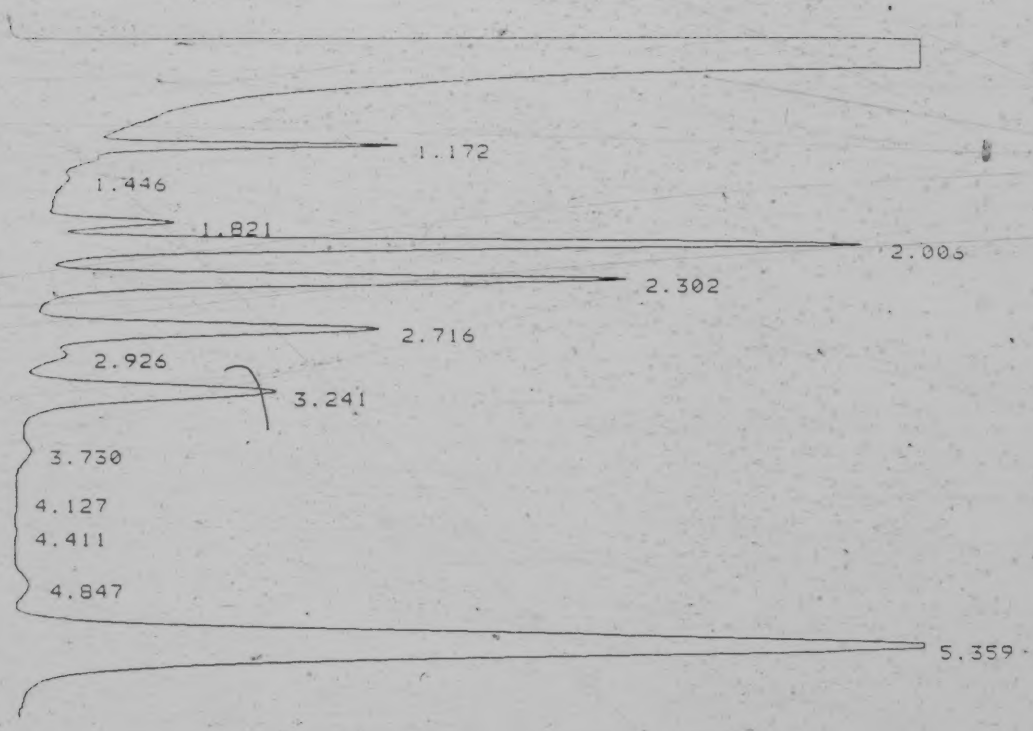
Parent[1]	Code[2]	Outcrossing rates (%) from Rakow and Woods (1987)[3]
A = Westar	(1982_C5)	50.0
B = DI820	(1982_C10)	10.0
C = 77-4397E	(1983_B2)	15.0
E = WW48-29-80	(1983_B7)	15.0
	(1983_B8)	55.0
	(1983_B9)	55.0
	(1983_C1)	5.0
	(1983_C6)	70.0
	(1984_A4)	7.7
	(1984_A6)	29.8
	(1984_B1	2.1
	(1984_B15)	38.3
	(1984_C14)	2.1
	(1984_C22)	29.8
	(1984_E3)	6.4
	(1984_E8)	28.3

Table 4.1. Outcrossing rate of 16 individual *B. napus* plants.

1 - Low erucic acid parental cultivar and lines used by Rakow and Woods (1987).
2 - Year of field testing in Saskatoon (Rakow and Woods, 1987) and parental code.
3 - Outcrossing rates of parental plants from Rakow and Woods (1987) experiment (unpublished). Outcrossing rates calculated as: [(number of seeds with erucic acid/total number of seeds) x 100].

In order to repeat the experiment of Rakow and Woods and determine the correlation between outcrossing rates from each line it was first necessary to isolate and increase low erucic acid S_1 seed sufficient for a field trial. To accomplish this objective, 20 seeds from each of the 16 lines were placed on filter paper soaked with distilled water in 100 x 15 mm petri dishes. When each seed germinated and the radicle had a length of about 10 mm, the outer cotyledon was removed and tested for percent erucic acid by gas

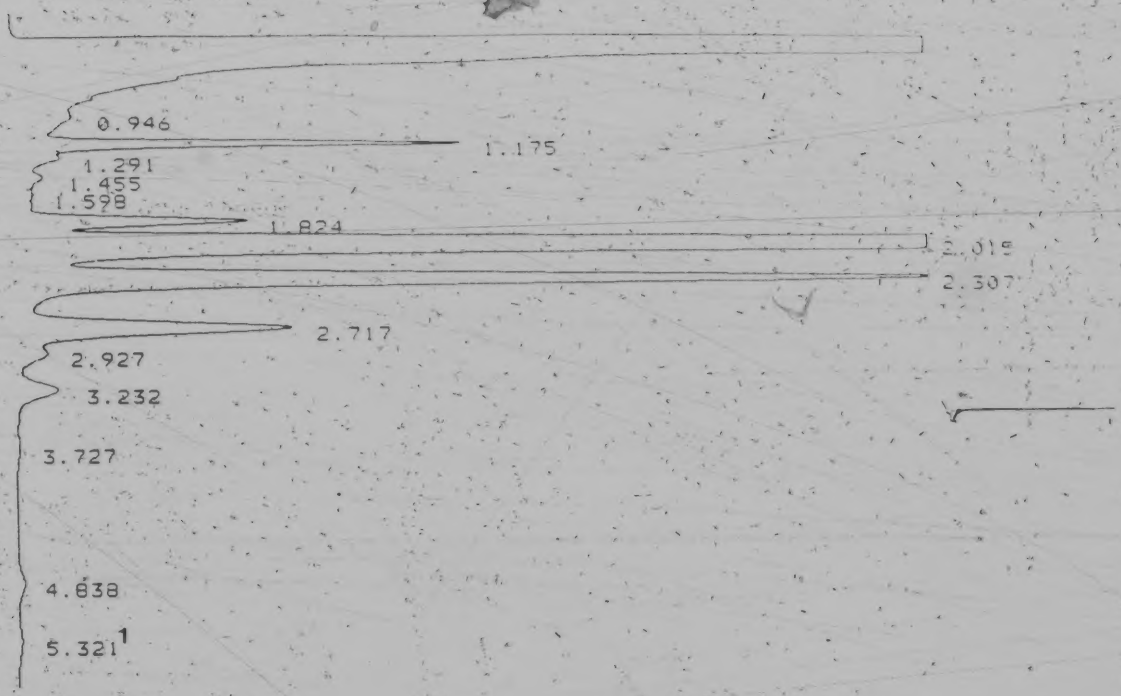
chromatography (GC) using the half-seed technique of Downey and Harvey (1963). Half-seeds were prepared for GC analysis using a modified version of the method described by Thies (1971). Half-seeds were dried, crushed in a 0.5 mL polypropylene microcentrifuge vial with the end of a glass rod, and treated with 0.2 mL of methylating solution (10 g sodium methylate + 600 mL methanol + 300 mL petroleum ether - Skelly B). Samples were vortexed for several seconds and transesterification (methanolysis) of the glycerides allowed to proceed at room temperature for 1 hour to give methyl esters of the fatty acids. An additional 0.2 mL Skelly B was then added and the mixture allowed to stand for 10 minutes, permitting methyl esters of the fatty acids in Skelly solution to separate from the methylating solution. One microlitre of the Skelly B layer (supernatant) was then injected into the GC. Gas chromatographic analysis of fatty acids was performed using a Varian 6000 gas chromatograph fitted with a flame ionization detector. The column used was a 2 m x 2 mm (ID) glass column packed with Supelco GP 3% SP-2310/2% SP-2300 on 100/120 chromosorb W AW. Oven temperature was held at a constant 240 °C with injection and detector temperatures set at 280 °C. Helium was used as the carrier gas. Seedlings with an intact embryo from which the outer cotyledon showed 1.0% or less erucic acid were refrigerated at 5 °C until all remaining seeds were tested. Seedlings with erucic acid contents greater than 1.0% were discarded. The distinction between the acceptable and unacceptable seeds was easily accomplished (see Figures 4.1, 4.2, and 4.3). Seedlings were planted on May 21, 1987 in the greenhouse. A mechanical failure generating a temporary heat surge killed many of the seedlings from this planting. The terminal raceme of each surviving plant was covered with a glassine bag which remained until cessation of flowering. Self pollination was induced by shaking the bag each day and selfed seed harvested at maturity.



CHANNEL NO: 1 SAMPLE: HERO METHOD: XTHESIS

PEAK NO	PEAK NAME	RESULT F.ACID	TIME (MIN)	AREA COUNTS	SEP CODE
1	C16:0	3.980	1.172	71251	BV
2	C18:0	2.207	1.821	39503	BV
3	C18:1	17.007	2.006	304433	VV
4	C18:2	13.642	2.302	244203	VV
5	C18:3	9.094	2.716	162791	VV
6	C20:0	8.186	3.241	146533	VV
7	C22:1	45.884	5.359	821355	VB
TOTALS:		100.000		1790070	

Figure 4.1 Gas chromatogram of the high erucic acid *B. napus* cultivar Hero (S82-4362) using a single seed. Note erucic acid ($C_{22:1}$) at retention time 5.359 minutes is 45.884% of total fatty acids. Also shown are the fatty acids: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), and arachidic ($C_{20:0}$).

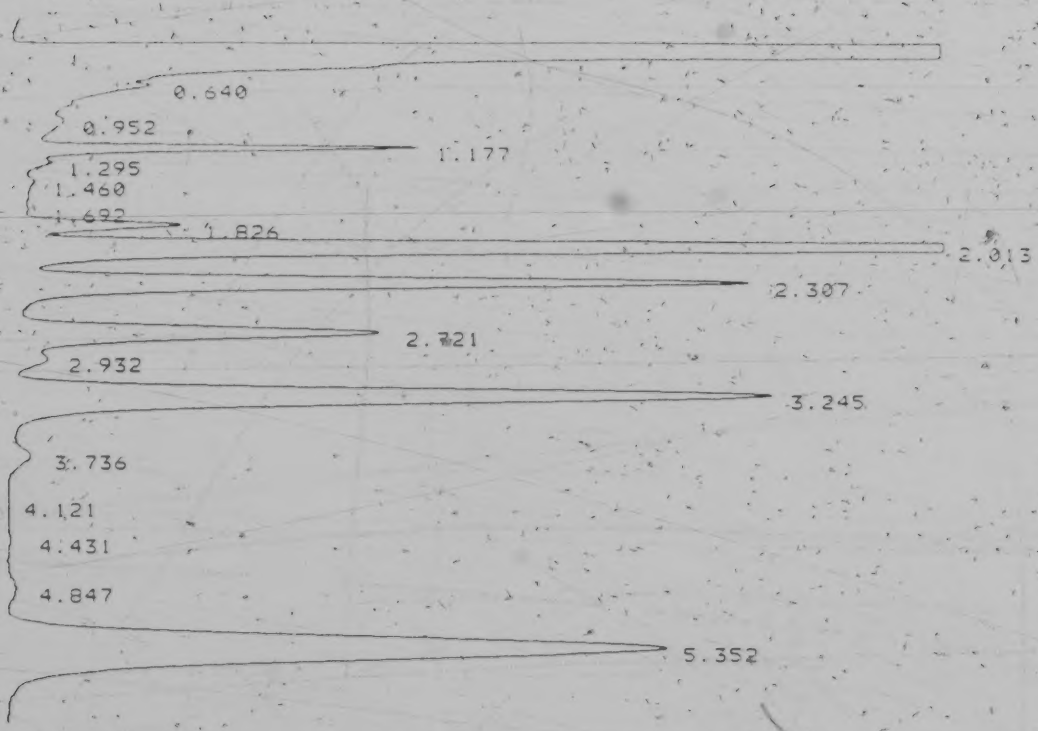


CHANNEL NO: 1 SAMPLE: WESTAR METHOD: XTHESIS

PEAK NO	PEAK NAME	RESULT F.ACID	TIME (MIN)	AREA COUNTS	SEP CODE
1	C16:0	5.485	1.175	94397	VV
2	C18:0	3.942	1.824	67838	VV
3	C18:1	59.679	2.015	1027090	VV
4	C18:2	22.281	2.307	383457	VV
5	C18:3	7.408	2.717	127492	VV
6	C20:0	1.206	3.232	20752	VB
TOTALS:		100.001		1721030	

Figure 4.2 Gas chromatogram of the low erucic acid *B. napus* cultivar Westar using a single seed. Note erucic acid ($C_{22:1}$) at retention time 5.321 minutes is <1.0% of total fatty acids[1]. Also shown are the fatty acids: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), and arachidic ($C_{20:0}$).

1 - Erucic acid retention time of 5.321 is visible in the gas chromatogram but not in the tabular summary since area peak counts ≤ 5000 were rejected.



CHANNEL NO: 1 SAMPLE: WESTAR*HERO METHOD: XTHE313

PEAK NO	PEAK NAME	RESULT F.ACID	TIME (MIN)	AREA COUNTS	SEP CODE
1	C16:0	3.968	1.177	84968	VV
2	C18:0	2.280	1.826	48822	VV
3	C18:1	24.853	2.013	532250	VV
4	C18:2	13.821	2.307	295993	VV
5	C18:3	7.933	2.721	169885	VV
6	C20:0	19.925	3.245	426713	VV
7	C20:1	0.470	3.736	10069	T
8	C22:1	26.751	5.352	572896	VB
TOTALS:		100.001		2141600	

Figure 4.3 Gas chromatogram from a single F_1 seed from the cross: Westar (low erucic acid cultivar) X Hero (high erucic acid cultivar). Note erucic acid ($C_{22:1}$) at retention time 5.352 minutes is 26.751% of total fatty acids. Also shown are the fatty acids: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic ($C_{20:0}$), and eicosenoic ($C_{20:1}$).

To compensate for lost plants, 10 whole seeds from each of the 16 lines were seeded May 29, 1987. Flowering plants were treated as described above. Twenty selfed seeds were bulk tested for percent erucic acid ($C_{22:1}$) content from each plant and low erucic acid (< 1% erucic) progeny retained for field testing. The procedure followed for 20 seed bulk erucic acid analysis was the same as the half-seed method except for larger reagent volumes and transesterification time. Seeds were crushed in 4 mL screw cap glass vials with a glass rod and vortexed after addition of 1.0 mL methylating solution. Samples were left for 30 minutes followed by addition of 1.0 mL Skelly B. Ten minutes later, 1.0 µL of supernatant was injected in the GC using conditions identical to those described for half-seeds. The second seeding was not sufficient to complete the requirements for low erucic acid S_2 seed so a third planting, October 24, 1987, using the half-seed technique, was necessary.

In addition to testing for low erucic acid S_2 seed (Figure 4.2), high erucic acid seed was tested by gas chromatography (Figure 4.1). The high erucic acid rape selected was line S82-4362 (subsequently licensed in 1989 and given the name 'Hero'; provided courtesy of P. McVetty, University of Manitoba). Twenty single seeds and one bulk 1.0 g sample of Hero was tested for percent erucic acid using the techniques described above. The objective of this preliminary seed lot analysis was two fold. First, the ability to easily distinguish S_3 seed from S_2F_1 (e.g. S_2 X Hero yielding an F_1 hybrid, but referred to here as the 'S_2F_1' hybrid) on the basis of the erucic acid biochemical marker would be directly proportional to percent erucic acid in the high erucic acid parent. If Hero proved to yield less than 40% erucic acid, then this line would be discarded in favour of an alternate high erucic acid line such as Reston. The randomly collected 1.0 g sample from the seed lot showed a 50.3% erucic acid content which was quite acceptable for the proposed field test. Second, the reason for

testing single Hero seeds was to establish if the line was heterogeneous for low erucic acid alleles since 'low erucic acid' Hero pollen crossed with the S_2 plants would result in an underestimation of outcrossing rates. All 20 Hero single seeds tested were high erucic acid phenotypes, with the lowest observed $C_{22:1}$ content of 46.3% and a mean of 52.3%. This sample size was too small to quantitate possible segregation for low erucic acid at the two multi-allelic rape erucic acid loci, but the random sample did suggest that heterogeneity for low erucic acid, if present, was infrequent. Banks (1988) detected 3 low erucic acid seeds in a sample of 107 Reston seeds and was unable to resolve if the Reston population was contaminated or polymorphic for low erucic acid. Controlled crosses were not attempted to establish the presence or absence of low erucic acid alleles in the Hero seed lot in this experiment.

On May 17, 1988, Hero was machine planted into Landry series soil at the Beaverlodge Research station (55° 13' N, 119° 24' W). The high erucic acid seed was drilled at a depth of 2.5 cm using a 6 row cone seeder and seeded at a rate of 1.5 g per 8 m row. Alternate cones were used in the seeder giving center blank rows with seeded rows 40 cm apart. Mono-ammonium phosphate (11-51-0) was drilled into every row at 67 kg ha^{-1} along with Furadan-10 granular (systemic insecticide containing 10% carbofuran active ingredient) at a rate of 2.7 kg ha^{-1}. Benolin-R (insecticide and fungicide dust contain 6% benomyl + 10% thiram + 50% lindane active ingredients) was mixed with each 1.5 g package of seed at a rate of 0.25 g $package^{-1}$ (approximately 5 times the standard application rate). Low erucic acid S_2 seed was rolled in Benolin-R and hand planted into the disk furrow 2.5 cm deep in the blank rows between Hero on the same seeding date as Hero (Figure 4.4). The S_2 seed was selected from 4 low erucic acid S_1 plants derived from each of the parental 16 lines giving 64 entries. The entries were seeded in a randomized complete-

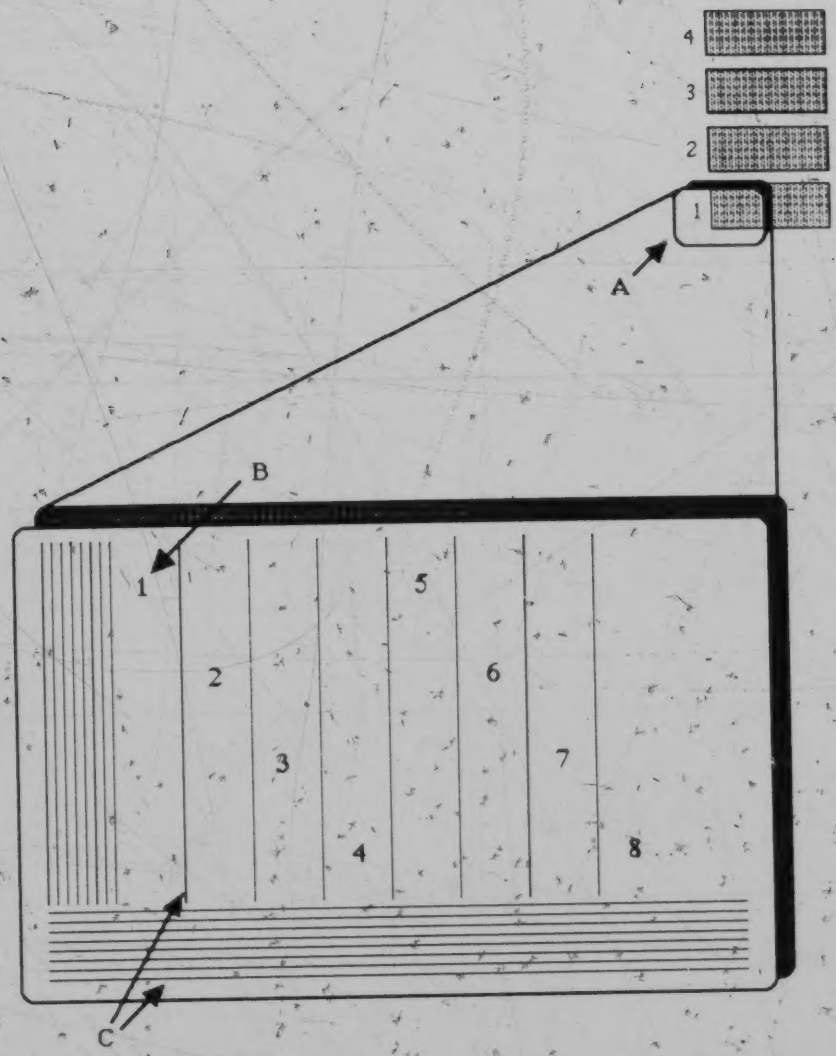


Figure 4.4 Seeding plan for *B. napus* outcrossing test. (A). One of 4 replications. A portion of replication 1 has been enlarged. (B). Low erucic acid plants 1 to 8 are shown. Each replication consists of 4 sibs from each of the 16 parents from Rakow and Woods (1987) giving a total of 64 plants per replication. Low erucic acid plants were seeded at least 1.5 m distant from the next nearest low erucic acid plant. (C). The long lines represent a single rows of the high erucic acid cultivar Hero. Eight guard rows were seeded on the perimeter of the site, and each low erucic acid plant was separate by a single 8 m rows of Hero on either side. All Hero rows were seeded 40 cm apart (diagram not to scale).

block design with 4 replications. Single plant plots were initially seeded with 4 seeds from each entry to ensure stand establishment, but were trimmed to 1 plant prior to flowering making 256 single plant plots (16 parents * 4 low erucic acid S_2 progeny plant from each parental line * 4 replications; Figure 4.4). Only one low erucic acid entry remained in each row prior to flowering and each entry was positioned at least 1.5 m from any other low erucic acid entry. The perimeter of the test was seeded with 8 additional 40 cm spaced rows of Hero to ensure uniformity of plot conditions.

Early flowers were removed from the low erucic plants to ensure coincident pollination with Hero. Similarly, remaining flowers from the low erucic acid entries were removed when Hero flowering was 70% complete. This ensured that each low erucic acid plant was able to randomly self-pollinate or outcross. Low erucic acid plants were hand harvested on September 20, 1988 (243 plants were harvested; the 13 missing plots were accounted for by absence of stand establishment in 9 plots and lack of coincident flowering with Hero in 4 plots).

Temperature, rainfall, and hours of sunshine for the months May through September, 1988 were near long-term averages. The mean temperature of 12.8 °C and total rainfall, 273.7 mm, were 0.4 °C and 28.3 mm higher respectively than the 73 year average recorded at the Beaverlodge Research station. Total sunshine for the 5 months, 1242.6 hours, was 31.5 hours lower than the recorded 66 year mean.

To determine the outcrossing rates of each plant using erucic acid as a marker, two methods have been employed. Seeds may be tested individually for presence or absence of erucic acid (Rakow and Woods, 1987; Banks, 1988) or bulk tested (Rudloff and Schweiger, 1985). Both of these methods were utilized to establish outcrossing rates, the first being single seed analysis.

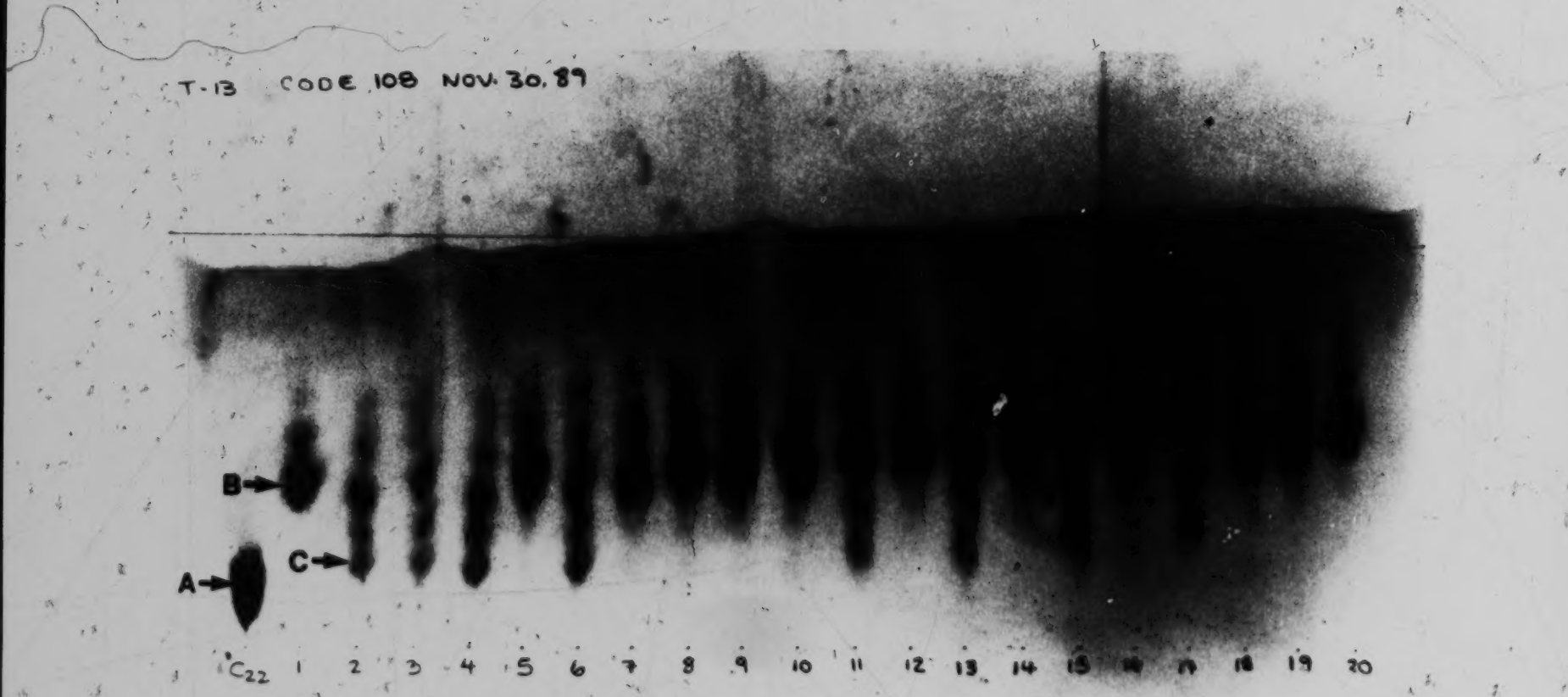
Presence or absence of erucic acid for 20 single seeds (S_2F_1 if outcrossed and S_3 if self-pollinated) were tested using the paper chromatography (PC) method described by Thies (1971). Single seeds were crushed in 0.5 mL polypropylene microcentrifuge vials. The crushed seed was then soaked in 100 µL of 5% potassium hydroxide in methanol and left uncapped at room temperature overnight allowing hydrolysis of fats to form free fatty acids. The dry residue was vortexed after addition of 100 µL of 1.5 N hydrochloric acid and 150 µL of Skelly B. Lacking the developing tank and sampling apparatus described by Thies (1971), sheets of Whatman No. 1 filter paper were cut to 12.5 cm in height by 23 cm in width to facilitate development in 500 mL Pyrex beakers. Chromatograms were developed using the reverse phase technique so, before placing samples on the paper, the paper was made non-polar by saturating each sheet with a solution of 10% paraffin oil in diethyl ether. Sheets were then extracted from solution and air dried. Two microliters of solution from each sample was placed at 1.0 cm intervals 2.0 cm from the base of the impregnated chromatography paper. Twenty-one samples were placed on each sheet: 20 samples and 1 control (erucic acid C22:1,cis 13; cis-13-docosenoic acid from Sigma). Papers were folded twice, between sample spots, to prevent contact with the beaker wall then placed into beakers carrying sufficient 95% acetic acid (mobile phase) to come just below the sample spots. Beakers were sealed with saran wrap and paper removed after the ascending mobile phase travelled 7 cm at room temperature. Papers were air dried overnight in a fume hood to permit complete evaporation of solvent. Dried chromatograms were immersed in a 1% solution of cupric acetate in water for 30 minutes, followed by removing excess reagent by rinsing for 2.5 hours in running water. Papers were dried overnight at room temperature, or dried in a convection oven at 105 °C for 5 minutes. Colour development of fatty acid

cupric salt spots was performed by dipping chromatograms into a 0.03% solution of dithio-oximide in ethanol for 30 to 60 seconds. As with GC analysis, the distinction between low and intermediate erucic acid levels levels were easily distinguished using paper chromatography (Plate 4.1).

The 20 S_3 seeds tested for erucic acid from each S_2 plant were classified as derived from self-pollination if erucic acid was absent on paper chromatograms, or derived from outcrossing if erucic acid was present (Plate 4.1). (See Figure 4.2 for comparison of Westar, and Figure 4.3 for comparison of the S_2F_1, Westar X Hero, hybrid using gas chromatography). Rates of outcrossing were calculated for each plant.

Determining the outcrossing rate of individual plants by single seed analysis is more accurate than bulk analysis, but also tedious and expensive. Rudloff and Schweiger (1985) reported that erucic acid content from bulk seed analysis was highly correlated with outcrossing rates. Specifically, Rudloff and Schweiger harvested 40 low erucic acid plants that had been geometrically seeded 2.5 x 3 m apart into a stand of the high erucic acid variety Sollux. The authors then tested 100 single seeds for presence or absence of erucic acid using gas chromatographic analysis. Rudloff and Schweiger calculated percent zero erucic acid seeds (variable y) and the mean erucic acid content for each plant (variable x) and found a correlation of $r = -0.98$ ($P < 0.001$) with the regression $y = 105.5 - 3.83x$. Assuming Rudloff and Schweiger's assumption that mean single seed erucic acid determinations accurately reflected bulk analysis (there is no reason to suggest this would not be valid), then this technique would be useful in the present study. Unfortunately, the sample size recommended by Rudloff and Schweiger, 1 g or larger, was not available for many of the harvested plants in this study. In an attempt to determine if a strong correlation could be established between erucic acid content from a small seed

Plate 4.1 Paper chromatogram showing fatty acid separation. A. Erucic acid control sample ($C_{22:1}$, cis 13; cis-13-docosenoic acid). Lanes 1-20 represent single seed fatty acid determinations collected from a low erucic acid *B. napus* parent permitted to freely outcross or self-pollinate in a block of high erucic acid plants. B. Lanes 1,5,7,8,9,10,12,14,16,18,19, and 20 are low erucic seeds, hence derived from parental self-pollination. C. Lanes 2,3,4,6,11,13,15, and 17 are intermediate erucic acid seeds, hence derived from cros-pollination of low and high erucic acid parents.


T-13 CODE 108 NOV. 30. 89
B
A
C
C22 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

sample and outcrossing rate, 20 seeds from each S_2 plant were bulk tested for percent erucic acid content using gas chromatography.

4.2 Results and Discussion

Analysis of outcrossing rates was made using Genstat V Release 4.04B developed at the Rothamsted Experimental Station, England. Analysis of variance was performed on the 4 replication randomized complete-block design using untransformed outcrossing rates (Table 4.2). Analysis indicates that the difference in outcrossing rates for parents (16 lines derived from 3 low erucic acid lines and the cultivar Westar) are highly significant ($P < 0.001$) but the interaction between S_2 plants and parents was not significant. The table of means is shown in Table 4.3.

The average rate of outcrossing ranged from 39.6 to 80.3 for the parents E3 and B7 respectively. Outcrossing rates for the 243 individual plants (Figure 4.5) ranged from 15.0% to 95.0% for E8 and B7 respectively. A wide range of outcrossing rates observed among individual plants within lines and cultivars has been reported. Olsson (1952) observed individual outcrossing rates of Lembke (Lenora) winter rape as low as 5-10% and as high as 80-85%. Rakow and Woods (1987) reported outcrossing rates for individual summer rape plants ranging from 2.1 to 75.0%. Using the low erucic acid cultivars Regent, Topas, Hanna, and Westar, Banks (1988) reported inter-plant outcrossing rates, for geometric planting, ranging from 0 to 100%. Comparison of geometric planting inter-plant outcrossing rates from three Canadian experiments is shown in Table 4.4.

Source of variation	D.F.	SS	SS%	MS	VR
Rep stratum	3	507.1	0.8	169.0	
Rep*units*1 stratum					
Parents	15	28475.3	45.4	1898.4***2	11.01
Parents.S_2	48	4816.6	7.7	100.3ns	0.58
Residual (Error)	176 (13)	30352.3	48.4	172.5	
Total	239	63644.2	101.4	266.3	
Grand total	242	64151.2	102.2		

Total number of observations is 256
Number of missing values is 13

Table 4.2 ANOVA table for rate of outcrossing of 16 *B. napus* lines at Beaverlodge.

1 - The individual samples, the rates of outcross in S_2 plants, and residual term are listed together under "units".
2 - *** and ns indicate significance at $P < 0.001$ and not significant respectively.

Estimated grand mean 55.3 ±6.6%

	Mean outcrossing rates					
	Siblings[2]					Rakow and
Parents[1]	1	2	3	4	Lewis[3]	Woods (1987)[4]
C5	53.8	56.3	49.9	55.0	53.7	50.0
C10	50.3	45.6	45.0	40.0	45.2	10.0
B2	55.0	62.5	68.8	57.5	60.9	15.0
B7	81.3	82.5	75.0	82.5	80.3	15.0
B8	68.8	75.0	69.4	72.5	71.4	55.0
B9	60.0	64.3	57.5	57.0	59.7	55.0
C1	42.5	47.9	47.5	50.0	47.0	5.0
C6	58.8	52.9	67.5	66.3	61.3	70.0
A4	55.5	50.3	53.8	50.0	52.4	7.7
A6	60.0	60.0	57.9	41.3	54.8	29.8
B1	55.0	63.9	51.3	51.3	55.4	2.1
B15	70.0	66.3	67.9	52.9	64.2	38.3
C14	51.3	48.8	48.8	46.3	48.8	2.1
C22	48.8	50.0	42.5	56.3	49.4	29.8
E3	38.8	38.8	34.6	46.3	39.6	6.4
E8	37.5	41.3	40.3	42.9	40.5	28.3
SED[5]	|←		9.3	→|	|←4.6→|	

Table 4.3 Table of means for rates of outcrossing.

1 - Sixteen low erucic acid parental lines.
2 - Four replication mean outcrossing rate for each of 4 selected low erucic S_2 plants.
3 - Average outcrossing rate of 16 parental lines (S_2F_1 vs. S_3 seed) in the present study.
4 - Average outcrossing rate of 16 parental lines (F_1 vs. S_1 seed) from Rakow and Woods (1987) duplicated from Table 4.1 for ease of reference.
5 - SED are not adjusted for missing values.

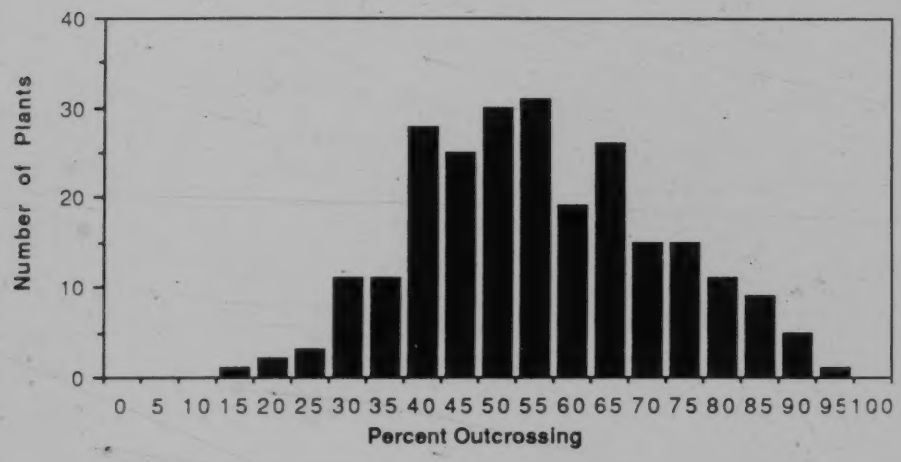


Figure 4.5 Percent interplant outcrossing observed in 243 plants of *B. napus* in 1988.

Author	Location	No. site years	% outcrossing
Rakow and Woods (1987)	Saskatchewan	3	21.8
Banks (1988)	Ontario	2	36.0
Lewis (current study)	Alberta	1	55.3

Table 4.4 Comparison of geometric planting outcrossing rates of *B. napus* from three Canadian experiments.

Mean outcrossing rates for the 16 parents in the current study exceeded Rakow and Woods (unpublished) outcrossing rates in all cases except for parent C6. The Mann-Whitney test was used to determine if the rates of outcrossing were significantly different in the two studies. Since $U_{calc\ 0.05,(2)\ 16,16} = 218 > U_{tab\ 0.05\ (2)\ 16,16} = 181$, the Mann-Whitney test indicated there was significantly greater outcrossing ($P < 0.001$) at the Beaverlodge location. To determine whether the outcrossing rates ranked in the same order in both locations the means were tested using the Spearman rank correlation and Kendall Tau B correlation procedures using SAS version 5.18. The Spearman correlation ($r = 0.53$) was significant ($P = 0.04$) while the Kendall Tau B correlation ($r = 0.36$) was not significant ($P = 0.06$). The lack of definitive rank correlation between the two sites does not fully endorse heritability of outcrossing rates consistent for each parent, but neither is heritability refuted. The environmental component in the Peace River Region may have influenced the outcrossing rate of the 16 parental lines (derived from the germplasm of 4 accessions: Westar, DI820, 77-4397E, and WW48-29-80) in a non-analogous manner than observed in Saskatchewan, thereby decreasing the rank correlation. The correlation between outcrossing rates would probably have been more definitive had 1 accession been used, rather than 4, and had the rape been tested at more than one site in the Peace River area.

The 20 seed S2 bulk tested erucic acid samples (x) were tested for correlation with percent low erucic acid samples (y) in the manner of Rudloff and Schweiger (1985). The analysis showed a correlation of $r = -0.55$ ($P < 0.001$) with the regression $y = 69.18 - 1.77x$. Although the correlation was significant, the degree of resolution necessary for useful comparison of the 16 parental lines grown in Saskatoon and the Peace River was lacking. These findings support Rudloff and Schweiger's claim that outcrossing rates can be

estimated by bulk erucic acid analysis, and that larger sample sizes (e.g. ≥ 1.0 g) are necessary to achieve a high correlation with outcrossing rates.

The high outcrossing rates and possible heritability observed in this study suggest exploitation of heterosis may be feasible by production of synthetics. Several authors have demonstrated that synthetics out-yield parents (Schuster, 1969, 1982; Schuster *et al.*, 1985; Schuster and Michael, 1976; Banks, 1988; Léon, 1987; Léon and Diepenbrock, 1987) and have suggested yield gains are attributable largely to genotypic combining ability, and partial outcrossing of autogamous rape. Heterosis may be maximized by selecting inbred lines, rather than cultivars *per se* (Brandle and McVetty, 1989ab) or using lines of diverse origin (Lefort-Buson and Dattee, 1982, 1985a; Grant and Beversdorf, 1985; Lefort-Buson *et al.*, 1987abc) to maximize general and specific combining ability. It would be interesting to determine if the high rates of outcrossing observed are unique to the Peace River region or an unusual single occurrence. Assuming the high rates of outcrossing are environment specific and not an anomaly, a two component synthetic with 50% outcrossing rates, rather than the norm of approximately 30% hitherto reported (where one-half of the crosses occur among plants of the same component and one-half between plants of different components) would produce progeny with 25% of the plants in the Syn-1 generation exhibiting the degree of outcrossing expected in F_1 hybrids.

5.0 Testing for residual sporophytic incompatibility in summer oilseed rape (*Brassica napus* L.).

There is no apparent reason why amphidiploid rape should be self-fertile, while the diploid progenetors are self-sterile, other than the initial need for primary colonization. Resynthesized rape may express SI in both A and C genomes (Hodgin, 1986), express self-sterility, or may be completely self-fertile (Olsson, 1960a). The S loci are not absent in the A and C genomes of rape but are largely inactive, although self-sterile rape has been reported in natural populations (Olsson, 1960a). Rape's inability to express SI can be overcome by introgression of SI from intraspecific and interspecific sources (Chapter 6) indicating that inherent S-alleles from either or both derivative genomes in rape may be present, but perhaps are not expressed due to interactions or changes to S-alleles, or to repression of the S-alleles by ancillary genes in the amphidiploid. Although the reason for polyploid *Brassica's* non-expression of SI remains an evolutionary mystery, it is possible that partial, yet incomplete, expression may be present, but is not detected by the usual self-pollination and fluorescent microscopy assays generally employed to detect the SI phenomenon. In this research, attempts are made to determine if partial or 'residual' SI exists in rape. To accomplish this objective, techniques were developed to measure crossing rates between parents.

5.1 Materials and Methods

The approach used to establish if residual incompatibility (RI) was present in rape was to measure seed set in reciprocally cross pollinated plants using mixed pollen from both parents. Rape varieties Westar and Reston were selected for these crosses since these cultivars have distinctly different erucic

acid ($C_{22:1}$) contents. Hybrids between these two varieties can be detected using gas chromatography. Westar has erucic acid contents of 0.3 to 0.4% total fatty acids in seed oil (Alberta Agriculture Agdex 149/33-10, 1982) while Reston has 40 to 48% erucic acid content (Alberta Agriculture Agdex 149/33-9, 1982).

5.1.1 High and low erucic acid crosses on a single stigma.

A preliminary test was conducted to determine if the unique silique physiology of rape could be utilized for RI analysis. The stigma of rape is bilobed and the silique is made of 2 carpels separated by a false septum giving two chambers. To establish whether or not pollination on one lobe was correlated with seed set in 1 carpel, single pistils were self- and cross-pollinated using erucic acid as a marker. This test utilized one Westar and one Reston plant grown in the greenhouse in the summer of 1986. When both plants were flowering, 10 buds were emasculated on the Westar plant. On each Westar stigma, one lobe was self-pollinated with a freshly opened Westar flower by touching a dehiscing anther lightly to the lobe. The remaining lobe was cross-pollinated in the same manner with Reston. No attempt was made to identify the valve of the silique in relation to the lobes. It was assumed that if a physiological barrier were present that prevented pollen tubes from randomly migrating down the pistil prior to syngamy, all seeds in one chamber would be either all low erucic acid (S_1) or all intermediate erucic acid (F_1). Siliques were harvested at maturity and the seeds from each chamber tested for erucic acid content (Table 5.0). Erucic acid analysis was discontinued after 3 siliques were tested since it was obvious that progeny seed in each chamber was the result of random pollination. Hence, this approach to determining RI was discontinued.

	Chamber A		Chamber B	
	No. crossed[1]	No. selfed[2]	No. crossed	No. selfed
Silique 1.	4	4	6	6
Silique 2.	7	3	10	7
Silique 3.	10	3	9	2

Table 5.0 Selfed and crossed seeds in individual chambers of *B. napus* when self-pollen was applied to one stigma lobe and non-self pollen was applied to the remaining lobe.

1 - Number of seeds with intermediate erucic acid levels indicating cross-pollination had occurred.
2 - Number of seeds with low erucic acid indicating self-pollination had occurred.

5.1.2 Reciprocal crosses using mixed pollen from both parents.

If RI does not exist in the varieties Westar and/or Reston, then one would expect no preferential fertilization by the pollen from either variety. Thus if a mixture of pollen from each variety was applied to a stigma, one would expect the ratio of self- to cross-fertilization to be as the two types of pollen in the pollen mixture. If any self- incompatibility was present there would be an increase in the proportion of crossed seeds. In a preliminary experiment designed to test these assumptions, 10 plants of each Westar and Reston were sown in the summer of 1986 in the greenhouse. Prior to flowering, main racemes of each plant were covered with glassine bags. Four pairs of simultaneously flowering Westar and Reston plants were paired and 10 to 12 mature buds on lateral branches were emasculated, and covered with glassine bags. Buds rather than open flowers were emasculated and crossed to avoid pollen contamination. However during flower development, the stigmas of *Brassicas* are initially self-compatible but become self-incompatible approximately 1 day prior to anthesis. For this reason, the emasculated buds were left uncrossed for 24 to 36 hours to allow maturity of the SI system, if present. Emasculated buds were then

pollinated by removing mature anthers from 10 flowers on the bagged main raceme of each parent, mixing the anthers in a 4 mL glass vial with a glass rod, then pollinating pistils of both parents in an alternate fashion one pistil at a time. An average of 10 pistils was pollinated on each plant by rubbing the glass rod across the stigma surface. Care was taken to ensure glass vials and rod were pollen free, sterilized with 95% ethanol, prior to anther collection and that the pollen was well mixed before pollination. Seed from the 4 pairs of reciprocally crossed plants was collected at maturity and tested for percent erucic acid content (Table 5.1). Chi square analysis indicates that no significant differences were observed between self- or cross-pollination of the reciprocally crossed Reston and Westar parents (Table 5.1).

Cross	Female parent	Progeny classes		Effective pollen ratio		χ^2
		Selfed	Crossed	Westar	Reston	
1.	Westar	4	8	9.06	13.94	0.36[1]ns[2]
	Reston	6	5			
3.	Westar	5	7	10.00	14.00	0.00ns
	Reston	7	5			
4.	Westar	6	6	11.00	13.00	0.18ns
	Reston	7	5			

Table 5.1 Frequency of self- and cross-pollinated progeny from reciprocally crossed *B. napus* cultivars Westar and Reston.

1 - Sample calculation for Chi square using cross 1 follows:

	Observed Ratio			Expected Ratio	
	Selfed	Crossed	%Westar	Selfed	Crossed
Westar	4	8	33.33	(.3939)(12) = 4.727	7.273
Reston	6	5	45.45	6.667	(.3939)(11) = 4.333
			Av.= 39.39		

$$\chi^2 = \sum \frac{(obs - exp)^2}{exp} = 0.355$$

For Westar, the effective pollen ratio is (4.727 + 4333) = 9.060; for Reston, the ratio is (7.273 + 6.667) = 13.940.

2 - ns indicates not significant at P = 0.05.

Analysis of reciprocal crosses involving plant 2 was discarded since crosses with Reston as the pistil parent yielded less than 1% erucic acid thus indicating the number 2 'Reston' plant did not possess the expected high erucic acid genotype. Another problem encountered was that it was difficult to distinguish between some selfed and crossed progeny on the Reston female parent. F_1 and S_1 seed was unambiguously determined from the Westar female parent which showed a range of erucic acid content from 0.0 to 28.5% with mean selfed values of 0.0% and mean outcrossed values of 22.0%. Erucic acid content of seed tested from the Reston female parents ranged from 15.0 to 41.0% and no clear inflection point was evident between selfed and outcrossed Reston progeny. Samples with less than < 28% erucic acid were treated as crossed and ≥ 28% as selfed. This classification was selected by plotting percent erucic acid values against seeds tested from the Reston parents and selecting the mid-point in the bimodal distribution but, again, the distribution was not clearly evident so some error in progeny classification is possible. The inability to distinguish progeny from the high erucic acid parent with certainty required further investigation before comments on RI would be meaningful.

The above experiment was modified and repeated. Prior to seeding Westar and Reston, the erucic acid status of the parents was confirmed using the half-seed technique, and Reston and Westar with erucic acid contents of > 40% and < 1% respectively were selected. Eight selected half-seeds from both Reston and Westar were sown in the greenhouse on October 27, 1986. The eight parents from each variety were paired and reciprocally crossed, harvested, and erucic acid contents determined.

Even with known parental erucic acid contents, distinction between selfed and outcrossed F_1 seed from Reston female plants could not be unambiguously determined (Figure 5.1). The point of inflection between selfed and outcrossed

rape appears to lie between 29 and 32% erucic acid. Using 29% erucic acid as the inflection point, 2 of the 8 reciprocal crosses are shown in Table 5.2. Although cross 2 shows a significant difference in seed set from reciprocal crosses, the difference is due to an excess of self-fertilization and therefore is not attributable to RI.

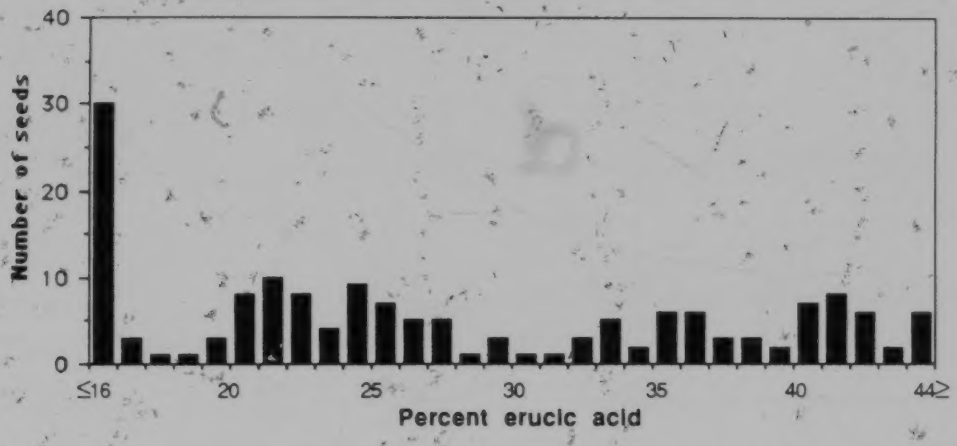


Figure 5.1 Percent erucic acid values for 159 F_1 seeds derived from a Reston parent pollinated with mixed Reston and Westar pollen.

Cross	Female parent	Progeny classes		Effective pollen ratio		χ^2
		Selfed	Crossed	Westar	Reston	
1.	Westar	55	28	89.80	42.20	0.32ns[1]
	Reston	14	34			
2.	Westar	15	14	55.12	84.88	8.93*
	Reston	81	30			

Table 5.2 Frequency of self- and cross-pollinated progeny from reciprocally crossed *B. napus* cultivars Westar and Reston.

1 - ns and * indicate not significant and significant at $P < 0.05$ respectively

The overlap in values of F_1 erucic acid contents less than and greater than mid-parent values for selfed and outcrossed Reston can be explained on the basis of environmental fluctuation and on segregation of erucic acid alleles at one or both loci. In reference to the latter, the dihybrid $E^{c1}e1E^{c2}e2$ would express near maximum erucic acid levels using the nomenclature of Krzymanski and Downey (1969). [Recall that Krzymanski and Downey (1969) assumed that the 5 alleles e, E^a, E^b, E^c, and E^d controlled synthesis of 0, 10, 15, 30, and 55 percent erucic acid respectively in the seed oil of *Brassicas*. Also, for the purposes of demonstration, ignore Chen and Heneen's argument (1989a) that E^a and E^c may be the same alleles]. Self-fertilization of the dihybrid could result in erucic acid contents ranging from 0 to 60% as could a cross between the dihybrid and a low erucic acid variety (e.g. $E^{c1}e1E^{c2}e2$ X e1e1e2e2). With the presence of multiple alleles, discussed earlier, the reasons for F_1 seeds with erucic acid contents less than or greater than the mid-parent values is obvious. $E^{c1}E^{c1}E^{c2}E^{c2}$ Reston would permit discrimination between S_1 and F_1 seed in the described mixed pollen studies, but isolation of such plants would require several generations of backcrosses and test crosses since numerous genotypes can express the same high erucic acid phenotype (ie. near maximum levels of erucic acid can be expressed by the genotypes $E^{b1}E^{b1}E^{b2}E^{b2}$, $e1e1E^{c2}E^{c2}$, $E^{c1}e1E^{c2}E^{d2}$, $E^{a1}E^{b1}E^{c2}E^{c2}$ etc.). This argument invalidates the effective use of high erucic acid parents of unknown genotype for detection of selfing and outcrossing on that parent. However, this does not invalidate the use of a high erucic acid line acting as the male parent for determining outcrossing rates in a zero erucic acid line. Since erucic acid is determined by gene interaction and multiple alleles at each of the two loci, selected high erucic acid plants would produce a negligible frequency gametes with low erucic acid alleles (previous GC testing of F_1 single seeds collected

from zero erucic acid lines that had been crossed with high erucic acid lines have consistently yielded erucic acid contents exceeding the low erucic acid parent). Thus, the ability to detect outcrossing in the low erucic acid parent is valid using the erucic acid marker.

5.1.3 Development of a high erucic acid, pale flower colour *B. napus*.

Rather than breed homozygous high erucic acid rape, a second unambiguous marker was sought for introgression into high erucic acid rape. The marker selected was a recessive pale flower colour marker. Rape plants bear lemon-yellow petals (wild type), pale yellow, orange, and pale orange petals which have the genotypes AABB, aaBB, AAbb, and aabb respectively (for discussion and references, see Séguin-Swartz, 1988). The objective was to introgress pale flower colour into a high erucic acid rape line and later, to reciprocally cross this line with low erucic acid wild-type flower plants using the mixed pollen technique. The ratio of self to outcrossed seed derived from the low erucic acid female parents could then be established by determining erucic acid contents via gas or paper chromatography. Self and outcrossed progeny from the high erucic acid-pale flower female parent could be determined by growing F_1 seed and later scoring for flower colour. Since the aaBB parent would be homozygous for recessive alleles at the A locus, all pale flower plants would be derived from self-pollination while yellow flower plants would be from cross pollination. Chen *et al.* (1988a) demonstrated that unlinked high erucic acid-pale flower plants are possible in the C-genome of *B. napus* suggesting the proposed technique is feasible. The procedure used to develop a high erucic acid, pale flower line of rape is shown in Figure 5.2.

Step 1 $P1^1$ aaBBe1e1e2e2 X AABBE1E1E2E2

↓

Step 2 F_1 AaBBE1e1E2e2 X AaBBE1e1E2e2

↓

Step 3 F_2 aaBB?1?1?2?2[2] X aaBB?1?1?2?2

↓

F_3

Figure 5.2 Protocol used to develop a high erucic acid, pale flower line of *B. napus*.

1 - aaBB - recessive pale flower allele; AABB - dominant (wild type yellow flower allele; E1, E2 - High erucic acid alleles at loci 1 and 2 respectively.
2 - Pale flower progeny selected and self-pollinated.

Eight S_1 Reston seeds from high erucic acid parents (determined using the half-seed technique) were sown on October 27, 1986. Sixty half-seeds were tested for percent $C_{22:1}$ in total fatty acids from 4 of these S_1 Reston plants. Five half-seeds with erucic acid contents greater than 40% were sown in the greenhouse on September 3, 1987 providing the high erucic acid and wild type flower colour P_1 for Step 1 (Figure 5.2). Pale-yellow petalled *B. napus* 'double haploid' seed was kindly sent by G. Séguin-Swartz from Agriculture Canada, Saskatoon (see Séguin-Swartz, 1988). Six seeds were sown on September 3, 1987 from which 4 plants exhibited the pale-yellow flower phenotype and the remaining 2 expressed the wild-type phenotype. To confirm that flower colour was determined by the genotype aaBB (Séguin-Swartz, 1988) each of the 6 plants were self-pollinated. Pale flower plants were also crossed with selected high erucic acid Reston (Step 1, Figure 5.2). All crosses were made by bud emasculation and pollination followed by bagging to prevent cross contamination. F_1 and S_1 seed was harvested, then seeded December 30, 1987 in the greenhouse. The expected frequencies of pale- and lemon-

petalled plants support Séguin-Swartz's (1988) observations that the double haploid pale-yellow petalled Westar plants are aaBB (Table 5.3). Erucic acid analysis on the 4 pale flower's S_1 seed was performed using bulk samples of 15 randomly selected seeds from each plant. In all cases there was less than 1% erucic acid thus supporting the origin from of each Westar and suggesting that the initial cross between Reston and the pale-flower rape was: aaBBe1e1e2e2 x AABBE1E1E2E2 as depicted in Figure 5.2 (Step 1).

Three plants each from 7 P_1 crosses (3 from Reston/Pale-Westar[1] crosses and 4 from the reciprocal cross) comprised the selected F_1 planting on December 30th. The main raceme of each plant was bagged just prior to flowering and induced to self-pollinate by shaking the bag once a day during flowering (Step 2, Figure 5.2). F_2 seed from each cross was harvested at maturity. This strategy was used rather than backcrossing to pale-Westar and

		Progeny classes		χ^2
Parents		Lemon[1] petal	Pale petal	Test Ratios
Westar-1 (Pale) selfed	(S_1)	0	15	0:1
Westar-2 (Lemon) selfed	(S_1)	15	1	3:1ns[2]
Westar-3 (Pale) selfed	(S_1)	0	20	0:1
Westar-4 (Lemon) selfed	(S_1)	9	4	3:1ns
Westar-5 (Pale) selfed	(S_1)	0	12	0:1
Westar-6 (Pale) selfed	(S_1)	0	17	0:1
Westar(Pale)/Westar(Pale)	(F_1)[3]	0	16	0:1
Westar(Pale)/Reston	(F_1)	21	0	1:0

Table 5.3 Segregation of pale yellow petaled Westar.

1 - Lemon petal represents the wild type flower petal in *B. napus*.
2 - ns indicates not significantly different at $P = 0.05$.
3 - Cumulative results from 2 pale yellow petal X pale yellow petal crosses.

[1] The slash between Reston and Pale-Westar indicates a cross using the convention of Purdy *et al.* (1968). This method of illustrating pedigrees is used throughout the text.

then selfing since the latter crosses would take longer to recover a high erucic acid genotype. By selfing the F_1, approximately 25% of the F_2 should show the pale flower phenotype. To get an approximate indication of the number of high erucic acid F_2 segregants, (again, ignoring multiple alleles known at the erucic acid loci), the 5 allele control model of Krzymanski and Downey (1969) was used. Selfing the F_1 according to this model would generate segregants 1:4:6:4:1 with approximately 0:10:20:30:40% erucic acid respectively. Since approximately 1 in 16 segregants exhibit the desired genotype for erucic acid and 1 in 4 the genotype for pale flower colour, then roughly 1 in 64 (1/4 x 1/16 = 1/64) F_2 segregants would be expected to have the desired genotype.

Rather than use the half-seed technique to test for high erucic acid phenotypes, selected F_2 seed was sown and first screened for pale flower colour. F_2 seed was selected from 4 selfed F_1 plants that exhibited vigorous growth and good seed set. (Note that selfing and selecting for high frequency of seed set put negative selection pressure on SI). To obtain at least 1 plant with both markers ($P < 0.05$), 256 F_2 seeds were sown (May 17, 1988) from each of the 4 selfed F_1 plants. Just prior to flowering, the main raceme was bagged, permitting self-pollination (Step 3, Figure 5.2). At flowering, all lemon colour plants were discarded and F_3 seed from all selfed pale-flower segregants harvested at maturity. The ratio of surviving pale-yellow petalled plants to lemon-yellow petalled plants is shown in Table 5.4. With the exception of the Reston-4/Pale-Westar-1 cross, the segregation ratios again support Séguin-Swartz's (1988) report that pale petal colour is determined by the aaBB genotype.

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Code	Parents	F_2 Progeny classes Lemon petal	Pale petal	Expected ratio 3:1 (χ^2)
I.	Reston-4/Westar-1 (Pale) selfed	67	8	8.2**1
II.	Reston-4/Westar-3 (Pale) selfed	19	6	<0.1ns
III.	Westar-1 (Pale)/Reston-4 selfed	36	19	2.7ns
IV.	Westar-5 (Pale)/Reston-4 selfed	58	26	1.6ns
Total		180	59	<0.1ns

Table 5.4 Segregation of F_2 populations for pale flower colour.

1 - ns and ** indicate not significant and significant at $P < 0.01$ respectively.

The frequency of high erucic acid seeds segregants in a population is positively correlated with the percent $C_{22:1}$ in a bulk sample (Chapter 4). In order to screen out undesirable low erucic acid material, 20 seeds were collected from each of the 30 F_2 plants and the seed from each plant bulk tested for percent erucic acid content using gas chromatography. From this analysis, 6 had erucic acid contents exceeding 30% C22:1. Half-seed F3 seed from the 6 F_2 plants revealed that the frequency of seeds exceeding 35% erucic acid was quite high and thus suitable as a source for high erucic acid, pale flower colour genotypes.

5.1.4 Reciprocal crosses between *B. napus* lines using mixed pollen from a pale flower-high erucic acid parent and a wild type flower-low erucic acid parent.

With the development of pale-yellow flower/high erucic acid lines, attempts to detect RI was ready to proceed. Low erucic acid (< 1.0% $C_{22:1}$), wild flower colour parents were sown at a rate of 6 seeds parent^{-1} in the greenhouse on June 27, 1989. The parents includedSC Global, Midas, Topas, and Westar;

and SI Midas*3/W8 and Global*3/W13 (SI line development is discussed in Chapter 7). The seed source for SC parents was from certified seed. Ten F3 high erucic acid (> 35% C22:1, again determined using the half-seed technique) pale flower seeds from selfed Reston-4/Westar-3 and Westar-1/Reston-4 (Codes II and III respectively in Table 5.4) were similarly sown on June 27th. The Midas*3/W8 and Global*3/W13 flowering plants were further tested for self-compatibility and self-sterility based on a pollen tube assay using fluorescent microscopy. The SC and SI lines and cultivars were reciprocally crossed with mixed pollen to the pale flower-high erucic acid plants and the seed from each cross was harvested at maturity. The crosses were performed as described earlier, with the only exception being that 3 pistils were pollinated on each plant rather than 10. In retrospect this change in technique was unfortunate since low seed set from many crosses necessitated discarding the cross. The poor seed set encountered for many of the crosses was surprising since earlier crosses using the same technique generally yielded 20 seeds silique^{-1} and no explanations are immediately obvious. Reciprocal crosses with Westar were repeated before termination of flowering to ensure sufficient seed set. On October 9, 1989 24 individual F_1 seeds collected from the successful crosses with pale flower-high erucic acid female parents were seeded in the greenhouse. Gas chromatographic analysis was performed on a maximum of 24 F_1 seeds from low erucic acid female parents crossed with mixed pollen. (Note: Chapter 4 showed a poor correlation between small bulk seed samples tested for erucic acid content and outcrossing rate, so this protocol was not attempted here).

5.2 Results and Discussion

The distinction between selfed and crossed progeny for low erucic acid SC and SI female plants based on erucic acid content of F_1 seed was unambiguous. The distinction between pale-yellow petalled F_1 and lemon-yellow petalled wild type flowers segregating from crosses with the high erucic acid pale flower female parent was similarly easily determined. The frequency of self- and cross-pollination for reciprocal crosses, and chi square analysis for each cross is presented in Table 5.5. The ability to detect self-incompatibility using the erucic acid and petal colour markers is clearly demonstrated with crosses involving SI and PSC parents (crosses 12, 13, and 14). Although crosses 2, 4, and 6 show significant differences in outcrossing rates between female parents, the deviation from expected values is due to a preponderance of self-fertilization (as in cross 2, Figure 5.2) and therefore not due to incompatibility. However, cross 10 exhibits significantly higher outcrossing than expected in both Westar-17 and III-3 suggesting RI may be present in one or either female parent.

The ability to detect preferential outcrossing using a biochemical (erucic acid) and morphological (petal colour) marker is supported by the analysis of reciprocally crossed *B. napus* using mixed pollen. This technique provides an alternative to seed set and fluorescent microscopy assays in determining self-incompatibility, but the ability to detect low levels of self-incompatibility (RI) was inconclusive. The ability to detect RI would be considerably enhanced by increasing the frequency of reciprocal crosses with the same pollen mixture and subsequent analysis of a larger sample. Also, in developing the pale-petal, high erucic acid line, two successive self-pollinations were used to derive the F3 seed (Table 5.2) selected for reciprocal crossing with the low erucic acid

Cross	Female Parent	Progeny classes[1] No. selfed	No. crossed	Effective pollen ratio Parent 1[2]	Parent 2	χ^2
1.	Global-27	16	6	23.49	11.51	0.50ns[3]
	II-1[4]	5	8			
2.	Midas-30	23	1	29.09	9.91	9.29*
	III-1	7	8			
3.	Topas-25	17	3	24.17	9.83	3.21ns
	II-2	6	8			
4.	Topas-31	24	0	35.55	10.45	13.53***
	II-3	10	12			
5.	Westar-2	11	13	23.00	25.00	0.08ns
	II-4	12	12			
6.	Westar-3	21	3	32.67	13.33	6.07*
	II-5	10	12			
7.	Westar-11	13	7	27.00	13.00	0.11ns
	II-6	6	14			
8.	Westar-13	4	2	16.71	10.29	0.26ns
	III-2	9	12			
9.	Westar-16	11	10	27.73	17.27	1.41ns
	II-7	7	17			
10.	Westar-17	4	20	15.16	31.84	5.22*
	III-3	12	11			
11.	Midas (SC)[5]	24	0	46.00	0.00	0.00ns
	III-4	0	22			
12.	Midas (SI)*[6]	1	23	13.24	33.76	13.38***
	III-5	11	12			
13.	Global (SI)[7]	0	15	16.52	21.48	29.23***
	II-8	3	20			
14.	Global (PSC)	5	19	19.98	24.02	10.73**
	II-9	6	14			
15.	Global (SC)	10	14	19.07	24.93	0.05ns
	III-6	11	9			

Table 5.5 Frequency of pale-petal vs. lemon-petal colour and low vs. high erucic acid contents in SC, PSC, and SI *B. napus*.

1 - Number of self- and cross-pollinations are based on single seed erucic acid determinations for first female parent and flower colour for second female parent.
2 - e.g. Parent 1 for cross 1 is Global-27 and Parent 2 for cross 1 is II-1
3 - ns, *, **, and *** indicate not significant, significant at $P < 0.05$, significant at $P < 0.01$, and significant at $P < 0.001$ respectively.
4 - The codes I, II, III, and IV, from Table 5.4, are pale flower-high erucic acid lines.
5 - SC, PSC and SI represent sporophytic compatible, partially sporophytic compatible, and sporophytic incompatible respectively.
6 - Midas(SI) and Midas(SI) are derived from Midas*3/W8 crosses (discussed in Chapter 7).
7 - Global (SI), Global (PSC), and Global (SC) are derived from Global*3/W13 crosses (discussed in Chapter 7).

lemon-yellow *B. napus*. As mentioned earlier, selection of high seed set from self-pollinated plants put negative selection pressure on sporophytic incompatibility. However, this selection pressure was not sufficient to suggest that the significant outcrossing rates observed for crosses 10, 12, 13, and 14 (Table 5.5) was due exclusively to the low erucic acid female parents. Inbreeding the pale-petal high erucic acid line for several more generations before repeating the reciprocal mixed pollen technique described would increase the confidence that observed increases in out-crossing is attributed exclusively to the low erucic acid female parent.

It is not known how much confounding effects, if any, can be attributed to environmental or mentor pollen effects. Regarding the latter, treated and untreated pollen or substances derived from pollen have overcome SI and GI (Nettancourt, 1977; Clarke and Knox, 1978; Stettler and Ager, 1984) yet little work has been done to quantify this influence in *Brassica* crosses using untreated pollen. Sarker *et al.* (1988) reported that pollen hydration and germination of SC pollen may occur adjacent to inactive intraspecific SI pollen on the same stigmatic papillae. These results show that the individual pollen grains behave independently which suggest that mentor effects in the mixed pollen experiment were not significant.

6.0 Introgression of sporophytic incompatibility into sporophytic compatible summer rape (*Brassica napus* L.)

In this research, attempts to introgress sporophytic incompatibility into *B. napus* L. ssp. *oleifera* (Metzg.) forma *annua* are are reported using crosses between: 1) *B. napus* and *B. campestris* 2) *B. napus* and *B. oleracea*, 3) *B. oleracea* and *B. campestris*, and 4) Swede and summer rape.

Brassica napus (rape, AACC) is generally self-compatible (Davey, 1938) while the diploids *B. campestris* (turnip rape, AA) and *B. oleracea* (cole crops, CC) from which amphidiploid rape is derived (U, 1935; Figure 2.1) are generally self-incompatible (Bateman, 1955; Thompson, 1957). Sporophytic incompatible *B. napus* has been developed by *de novo* synthesis of rape through hybridization of *B. oleracea* with *B. campestris* and doubling the chromosome number of the amphihaploid (reviewed by Namai *et al*, 1980). Similarly, interspecific crosses between *B. campestris* and *B. napus* have led to lines of SI rape (Mackay, 1973, 1976, 1977; Banks, 1988; Gemmel *et al.*, 1989). Self-incompatible Swedes have also been used as a source for introgression of S-alleles into rape (Banks, 1988, Gemmel *et al.*, 1989; Gowers, 1989). It is also possible to introgress S-alleles from *B. oleracea* into rape but the cross is so difficult that *de novo* synthesis of *B. napus* from the cross *B. campestris* X *B. oleracea* using embryo culture (McNaughton, 1963) is preferred for transferring *B. oleracea* genes into *B. napus*. Many of the S-alleles in Swedes were derived from ancestral resynthesized *B. napus* (Olsson, 1953, 1960a; Gowers 1974, 1975, 1979, 1989; Gemmel *et al.*, 1989). Resynthesized rape and Swedes range from fully self-fertile to completely self-sterile (Olsson, 1953, 1960; Mackay, 1977) suggesting that the genotype of the plant, and not the state of ploidy, determines self-fertility.

6.1 Materials and Methods

B. oleracea PGR 4130, *B. campestris* cv. Tobin and *B. napus* cv. Westar were sown in the greenhouse on December 6, 1985. The three species were crossed in diallel by bud pollination (PGR 4130 did not require vernalization to induce flowering) and covered with glassine bags to ensure crosses were not contaminated. F_1 seed was collected at maturity and 8 seeds from each successful cross was planted June 19, 1986. All F_1 plants were confirmed to be hybrids on the basis of visual inspection of F_1 plant morphology (cytological or isozyme confirmation was not attempted) and compared with the parents. Hybridization between PGR 4130 and Tobin or Westar was not successful while interspecific hybridization of *B. napus* and *B. campestris* was easily accomplished (Table 6.1), thus confirming previous reports (Palmer, 1962; Namai *et al.*, 1980).

	No. of flowers pollinated (A)	No. of hybrids obtained (B)	(B)/(A)
Westar/Tobin	136	1303	9.6
Tobin/Westar	100	682	6.8
Westar/PGR 4130	123	0	0.0
PGR 4130/Westar	105	0	0.0
Tobin/PGR 4130	103	0	0.0
PGR 4130/Tobin	153	0	0.0

Table 6.1 Seed set from reciprocal crosses between *B. campestris*, *B. napus*, and *B. oleracea*.

Although embryo rescue culture techniques have significantly increased the number of surviving hybrids (McNaughton, 1963; McNaughton and Ross, 1978; Namai *et al.*, 1980; Olsson and Ellerström, 1980; Prakash and Hinata, 1980; Hodgkin, 1986; Chen *et al.*, 1988b; Quazi, 1988; and references therein), these techniques were not attempted. Several hundred reciprocal crosses between

B. oleracea PGR 4123 and SC rape (Westar, Global, 86-411-6, Topas, and Midas) from a June 16, 1989 planting also failed to produce hybrids. When the latter crosses failed, further crosses with *B. oleracea* were not attempted.

Many of the Tobin X Westar F_1 seeds were shriveled whereas the Westar X Tobin seeds were generally smaller than produced by both parents, but appeared fully developed. Germination trials were not performed to establish viability, but the data (Table 6.1) is in agreement with the fact that crosses with *B. napus* as the female parent are more successful (Olsson and Ellerström, 1980). Four of the F_1 hybrids were backcrossed to Westar by bud pollination and the usual precautions to avoid contamination by cross pollination. The various crosses and ratio of flowers pollinated to seed set is shown in Table 6.2.

Female parent	Male parent	No. of flowers pollinated (A)	No. of hybrids obtained (B)	(B)/(A)
Westar/Tobin (A)	Westar	4	14	3.5
Westar/Tobin (B)	Westar	4	6	1.5
Tobin/Westar (C)	Westar	9	1	0.1
Tobin/Westar (D)	Westar	5	0	0.0
Westar	Westar/Tobin (A)	6	6	1.0
Westar	Westar/Tobin (B)	5	22	4.4
Westar	Tobin/Westar (C)	4	0	0.0
Westar	Tobin/Westar (D)	6	0	0.0

Table 6.2. Seed set for sesquidiploids reciprocally backcrossed to *B. napus* cv. Westar.

Although the low frequency of crosses does not allow definitive comparisons of data between Westar/Tobin and Tobin/Westar crosses, the data shows that the unbalanced F_1 sesquidiploid hybrids ($2n = 3x = 29$ AAC) are not completely sterile. To demonstrate that S allele(s) can be successfully introgressed from *B. campestris* into *B. napus* 6 Westar*2/Tobin seeds were sown in the greenhouse on June 27, 1989. Each plant was self-pollinated to

determine if sporophytic incompatibility was present. Self pollination was performed by bagging the main raceme just prior to flowering then actively self-pollinating freshly opened flowers by touching the stigma of target pistils with a dehisced anther from mature flowers removed from the same raceme. Three selfed pistils were randomly collected 24 hours after pollination and tested for pollen tube penetrations into stigmatic papillae using UV florescence microscopy. The remaining selfed flowers on the bagged raceme were left to develop seed. The hybrid plants were also used as pollen parents and crossed with Westar to give a measure of pollen fertility (Table 6.3) rather than use synthetic pollen germination media or testing viability by techniques such as iodine staining.

BC_1F_1 Plant	Self pollinated BC_1F_1 plants			Westar/BC_1F_1[1]	
Westar*2/Tobin(B)	No.flowers pollinated	No. seeds obtained	Av. No. pollen tubes pistil-1	No.flowers pollinated	No. seeds obtained
B1	5	46	+[2]	4	32
B2	5	1	4.7	4	25
B3	5	0	1.3	4	0
B4	5	0	2.0	3	6
B5	5	23	+	4	17
B6	5	1	2.7	4	18

Table 6.3 Seed set and pollen tube counts for BC_1F_1 rape.

1 - BC_1F_1 plants were used as pollen parents and crossed with Westar to give a measure of pollen fertility.
2 - Values for pollen tube counts are the mean of 3 observations. Plus (+) indicates that 100 or more tubes pistil-1 were observed.

Although the pollen fertility is low as indicated by the Westar X BC_1F_1 crosses, selfed seed set and pollen counts suggest plants B2, B4, and B6 are SI while plants B1 and B5 are SC (Table 6.3). Plant B3 and possibly plant B4 are

questionable since low pollen fertility prevented seed set in the female (Westar) parent. Low seed set due to unbalanced microspores is not unexpected. Variable seed set resulting from gamete selection pressure in similar crosses with allotriploids (*B. napus*-*B. campestris* crosses) was demonstrated by Mackay (1977) who showed that balanced gametes (n = 19) and unbalanced gametes (n = 10, 11, 17 and 18 were most effective in fertilization. Had the objective been to breed balanced lines of SI rape with introgressed *B. campestris* S alleles, fewer backcrosses would be required than introgressing an analogous dominant gene in diploid crosses since the F_1 allotriploid (AAC) contains 2/3 of the recurrent parents' germplasm (Mackay, 1977). The most expedient method of introgression and backcrossing *B. campestris* genes into *B. napus* has been reviewed (Mackay, 1977; Gowers, 1982).

In an attempt to identify SI rape with *B. campestris* background, 8 populations of rape plus a Westar check were screened for SI. The populations of rape were selected from part of an ongoing breeding program at the Agriculture Research Station in Beaverlodge, Alberta. Forty seven lines were selected from the 8 populations. Each line consisted of *B. napus* X *B. campestris* hybrids which had been backcrossed to *B. napus* for two generations (*B. napus*/*B. campestris*/2/3*B. napus*). Six seeds from each of the 47 lines and the Westar check were seeded in the greenhouse on December 17, 1986. Just prior to flowering, the main branch on four plants from each line was covered with a glassine bag to prevent cross pollination. Each day, the bag was shaken to allow pollination within the bag. The remaining two plants from each line were not bagged and induced to cross pollinate with surrounding rape by sliding pollen covered hands from plant to plant. The outcrossed plants were used as checks to visually compare seed set with selfed (bagged) plants at maturity. The Westar check was treated in the same manner. The

expectation was that selfed SI plants would exhibit low seed set while outcrossed plants would show 'normal' seed set (normal meaning siliques filled with seed as usually encountered with SC rape under greenhouse conditions). Self-compatible plants were expected to exhibit normal seed set for both selfed and outcrossed plants. All plants within and between the 47 lines and the Westar check were visually scored for seed set at maturity. The SC Westar control showed no difference between selfed and outcrossed plants as expected. However, none of the 47 lines showed differences between selfed and outcrossed plants which was not expected. Differences between lines were apparent but no lines exhibited the reduced seed set expected of sporophytic incompatible rape. F_1 sesquidiploid hybrids (2n = 3x = 29, AAC) hybrids from the initial cross between *B. napus* and *B. campestris* are expected to be functionally SI. The *B. campestris* S-allele in the sesquidiploid behaves as a dominant allele in the new genetic background, and since the SI S-allele is from the A genome of *B. campestris*, the plants are diploid at the S locus (Mackay, 1977). Assuming all 47 lines were true hybrids, BC_1F_1 plants would be expected to segregate 1 SC : 1 SI. Had random selection of BC_1F_1 plants occurred, 25% of the BC_2F_1 plants should be SI, or approximately 70 SI plants from the 282 plants grown representing the 47 lines. Since no SI plants were detected in BC_2F_1 plants, it is likely that SC BC_1F_1 plants were strongly selected. Although weakly SI lines may not have been detected by visual screening of seed set, it is unlikely these genotypes played a significant role in this test due to the uniformity of observations.

The final method attempting to introgress S-alleles into rape utilized several commercially available Swede cultivars. Swedes (Canadian), also called rutabaga (American), are known to possess S-alleles, derived largely from ancestral resynthesized *B. napus*. To facilitate this objective, the commercial

Swede rapes: Laurentian (Fortune), Purple Top Laurentian, Purple Top White Globe (Navet), and Early Snowball (see Appendix A for seed sources) were sown in the greenhouse on June 16, 1988. Inbred rape lines 86-411-6, Global, Topas, Westar, and Midas were seeded 6 weeks later under similar conditions to provide the SC rape source. Swedes were induced to flower by vernalization at 4 °C for 80 days with 8/16 hour periods of light/dark, then returned to normal greenhouse conditions with the SC rape. Flowering Swedes were tested for self-incompatibility using the pollen tube assay before reciprocally bud crossing with SC rape. No fertility barriers exist between Swedes and rape so most crosses were successful. The cultivar Purple Top Laurentian was omitted from these crosses since coordinated flowering could not be achieved. F_1 seed was harvested at maturity and selected reciprocal hybrids were seeded June 27, 1989 in a greenhouse. These selected hybrids were: 'Westar X each Swede line' and 'Fortune X each SC line' (Table 6.4). Vernalization was not required to induce flowering for most plants, suggesting that the summer habit is dominant. Self-fertility of hybrids was tested using fluorescent microscopy. Due to variation in F_1 seed set, germination, and flowering, it was not possible to test all hybrids for sporophytic incompatibility. No attempt was made to induce flowering, and plants not flowering by October 15, 1989 (108 days) were discarded.

6.2 Results and Discussion

Segregation for total SC and SI (Table 6.4) plants was 25:27 respectively which was not significantly different from a 1:1 ratio ($P < 0.75$) suggesting the Swedes were not homozygous for SI at either the derivative *B. campestris* (AA) or B. oleracea (CC) loci. The results also demonstrate that SI can easily be introgressed into rape from Swedes.

Code	Cross	Number of SC plants	Number of SI plants
I.	Laurentian (Fortune)/Westar	2	1
II.	Westar/Laurentian (Fortune)	4	-
III.	Early Snowball/Westar	1	9
IV.	Westar/Early Snowball	1	6
V.	Navet/Westar	-	2
VI.	Westar/Navet	3	4
VII.	Laurentian (Fortune)/86-411-6	-	-
VIII.	86-411-6/Laurentian (Fortune)	-	-
IX.	Laurentian (Fortune)/Global	1	3
X.	Global/Laurentian (Fortune)	1	-
XI.	Laurentian (Fortune)/Topas	-	-
XII.	Topas/Laurentian (Fortune)	3	1
XIII.	Laurentian (Fortune)/Midas	4	-
XIV.	Midas/Laurentian (Fortune)	5	1

Table 6.4 Reciprocal F_1 hybrids (*B. napus* ssp. rapifera X *B. napus* ssp. *oleifera*) tested for sporophytic compatibility and sporophytic incompatibility.

The allelic relationships between selected hybrids, SI rape plants T (Midas*3/T11), W (Midas*3/W8), and Westar was investigated. T and W have different S-alleles (discussed in chapter 7) whereas Westar is SC. Selected F_1 hybrid plants coded III-1, III-6 (plant 1 and plant 6), IV, V, VI, IX (Table 6.4) as well as single plants of Westar, W, and T were crossed in diallel. The crossing protocol was to emasculate buds just prior to flowering, bag the pistils for 24-36 hours until the pistil matured to the point normally encountered at flowering (allowing the SI mechanism to become fully functional), then rubbing dehisced anthers onto the stigmatic surface. Glassine bags were again placed over the pollinated pistils until removed 24 hours later, at which time pistils were tested for pollen compatibility using fluorescence microscopy. At least 6 pistils were assayed for each determination. Male and female plants with different S-allele(s) were expected to show a large number of pollen tube penetrations (SC cross) while plants with the same S-allele(s) were expected to display

diminished penetration in at least one of the reciprocal crosses (SI cross), depending on S-allele dominance relationships in the stigma or pollen if present. Westar was used as a control to ensure fertility of hybrid F_1 plants and W and T lines. In all cases, crosses with Westar in either direction gave 100 or more pollen tube penetrations into stigmatic papillae indicating all crosses were fully compatible and that pollen or pistil fertility in the hybrids, W and T plants was sufficient to allow meaningful collection of data (Table 6.5). Crosses revealed that T and W possessed different alleles, and also not shared by any of the hybrids tested. At least 4 S-alleles were detected in the F_1 rape-Swede hybrids. Codes III-1, III-6, and IV shared a common S-allele while V and IX were unique. VI appears to share a common allele with IV, but not with III-1 or III-6. Reciprocal crosses between IV and VI were repeated several times, and each time pollen tube penetrations pistil^{-1} were less than expected (< 100 pollen tubes) for full fertility. Since VI does not share IV's common S-allele with III-1 and III-6, several explanations are feasible. Assuming the results were indeed a sporophytic incompatibility phenomenon and not an unrelated artifact, the lower pollen tube penetrations from reciprocal crosses between IV X VI may indicate the both parents share a common S-allele, but on the other locus (e.g. on the A genome rather than the C genome) which is not shared by III-1, and III-6. Since rape is SC, this would suggest that either both alleles were derived from the Swede (one S-allele from each of the derivative A and C genomes) or that the interaction between the genes from the Swede and rape induced an inactive S-allele in rape to be expressed by removal of a repressor, change in an epistatic modifier etc. The former explanation is possible if the Swedes have synthetic rape ancestry and the latter explanation has merit (Hinata *et al.*, 1983; Thompson and Taylor, 1971; Nasrallah, 1974) but has not been well documented.

Pollen parent	Pistil parent								
	Westar	III-1[1]	III-6	IV	V	VI	IX	W	T
Westar	+[2]	+	+	+	+	+	+	+	+
III-1	+	2.0	0.5	2.3	+	+	+	+	+
III-6	+	0.2	3.2	0.7	+	+	+	+	+
IV	+	0.3	0.3	0.4	+	29.0	+	+	+
V	+	+	+	+	2.2	+	+	+	+
VI	+	+	+	43.3	+	5.7	+	+	+
IX	+	+	+	+	+	+	2.2	+	+
W	+	+	+	+	+	+	+	1.3	+
T	+	+	+	+	+	+	+	+	7.7

Table 6.5 Pollen tube counts from reciprocally crossed F_1 rape/Swede hybrids.

1 - Codes from Table 6.4.
2 - + indicates an average of 100 or more pollen tube penetrations stigma^{-1} were observed.

The aforementioned crosses demonstrate that SI alleles from *B. campestris* and Swedes can be easily introgressed into summer rape. Dominant SI progeny can be quickly screened using a pollen tube assay, and allelic relationships determined by a diallel crossing of SI plants. Also, SI can apparently be lost or selected out in only a few generations.

7.0 Development of sporophytic incompatible lines of summer oilseed rape (*B. napus* L.).

The objective of this research was to: a) introgress SI into domestic summer rape cultivars, b) examine the heritability of SI, c) develop near isogenic lines of SC and SI rape, and d) test agronomic performance of SC and SI lines under field conditions.

7.1 Materials and Methods:

Rapeseed germplasm used for this study included the two SI lines T2/2 * RC (T) and W1/2 * RC (W), courtesy of P. Banks (see Appendix A for pedigrees) reported to be segregating 1:1 for SC:SI. Parental SC sources included the breeder line 86-411-6, and the cultivars Global, Midas, Topas, and Westar. The SC rape lines were chosen on the basis of their divergent backgrounds (see Appendix A for origins). To confirm that the S-alleles for W and T were segregating 1:1 for SC and SI (P. Banks, personal communication), and to introgress sporophytic incompatibility into the sporophytic compatible material, the SC and SI lines were seeded in the greenhouse. T and W SI lines were placed in isolation to prevent cross pollination. The SI lines were planted on two dates as insurance against total loss due to unforeseen circumstances. Fifteen seeds of each T and W were seeded on May 21, 1987 followed by 8 T and 5 W seeds sown on September 3, 1987, which exhausted the seed sources. To ensure an overlap in flowering periods, the SC lines were seeded at 4 day intervals beginning 4 days prior to the May 21st seeding of T and W. Flowering times suitably overlapped when SC and SI lines were seeded simultaneously in May, so 6 seeds of each SC line were planted with T and W lines on September 3, 1987 with interval seeding omitted.

For May seeded lines, just prior to flowering the terminal racemes of T and W plants were covered with glassine bags. Each plant was mechanically self-pollinated by shaking the bag vigorously each day. The remaining flowers within each of the two segregating SI populations were outcrossed to maintain the source of SI, and to use as a comparison for seed set with the selfed terminal raceme. Seed set from selfed and outcrossed pistils was visually accessed for incompatibility. Plants showing poor selfed seed set and good outcrossed seed set were recorded as SI. Plants expressing no difference between selfed and outcrossed seed set were recorded as SC.

For September planted T and W lines, 10 pistils on the terminal racemes were self-pollinated by removing a flower with mature anthers and coating the stigma surface of open flowers with pollen 0 to 36 hours after bud opening. Self pollination was followed by cross pollination of 10 open flowers on the same terminal raceme using a mixed pollen source from the SC lines. Each pistil was selfed or crossed 0 to 36 hours after initial flowering. Selfed and crossed flowers were covered with glassine bags to prevent unwanted pollination. Three to 5 flowers opened each day requiring 2 to 4 days for self-pollination. Once selfing was completed, crossing was discontinued for 1 day. On the following day, all flowers of the previous day were removed, then cross pollination was initiated, taking another 2 to 4 days to complete. The removed flowers between selfed and crossed pistils permitted a convenient gap for tagging, separating selfed and outcrossed siliques thereby minimizing pollen contamination, and left a workable boundary between the two groups which simplified harvest. The entire procedure was normally completed before 30 flowers opened on the main raceme.

The total number of seeds silique^{-1} was tabulated at maturity for selfed and outcrossed pistils. Seed set from self bud pollination, which bypasses the SI

response (Shivanna *et al.*, 1978), could also have been used to compare with seed set from self-pollinated pistils, but was discarded in favour of the more rapid outcross method. The distinction between SC and SI plants for September seeded T and W lines was defined as the quotient of selfed (sl) and outcrossed (cr) plants where plants with sl/cr of ≤ 0.5 were considered SI, and all remaining plants were classified as SC. Given the small sample size this distinction between SI and SC plants did not reflect discrete boundaries, but enabled selection and comparison of progeny segregating for SI. The total number of SC and SI plants which survived from both May and September plantings for the T and W lines segregated approximately 1:1 as expected (Table 7.1).

	Progeny Classes		Expected ratio (χ^2)
	SC	SI	1:1[1]
T	8	5	0.7ns[2]
W	8	6	0.3ns

Table 7.1 Segregation of 2 lines of *B. napus* for sporophytic compatibility and sporophytic incompatibility.

1 - Assumes a one-locus model with dominance.
2 - ns represents not significantly different at P = 0.05.

From the May seeded lines segregating for SI, 5 plants exhibiting visually apparent differences between selfed and outcrossed seed set were selected for further analysis (3 W plants: W3, W8, and W13; and 2 T plants: T7 and T11). Prior to termination of flowering, mixed pollen from the SC lines was used to pollinate 5 pistils of each of the 5 selected T and W parents. Siliques from these crosses were harvested and, to confirm each parent was exhibiting sporophytic incompatibility, 20 F_1 seeds from each cross were seeded in the greenhouse

on September 3, 1987. For each F_1 plant, seed set from 10 selfed and 10 outcrossed pistils, as described for the September seeded T and W, was used to determine whether or not SI could be detected. From the 98 surviving F_1 plants tested, the total number of seeds silique^{-1} for outcrossed and selfed terminal inflorescence is shown in Figure 7.1 and Figure 7.2 respectively.

Outcrossed pistils (Figure 7.1) and selfed pistils (Figure 7.2) of T and W plants segregating for SC and SI had an average of 20.80 and 12.67 seeds silique^{-1} respectively. Plants with self-pollinated pistils yielding 20 or more seeds silique^{-1} were designated as SC. This was based on the average seed set (20.80) obtained for outcrossed pistils (and is also consistent with the seed set designation used by Gemmell *et al.*, 1989). Seed set for self-pollinated pistils (Figure 7.2) shows the SI and SC progeny classes. No definitive boundary separates the two classes but note the large frequency of siliques with no seed set. The frequency of 0 and low seed set silique^{-1} from selfed pistils decreases until the 5 seeds silique^{-1} class. This inflection point was deemed to be a useful boundary, defining SI plants as those with an average of 5 or less seeds silique^{-1}. To categorize all plants into progeny classes on the basis of seed set a quotient index was designed based on 5 seeds silique^{-1} or less for selfed pistils of SI plants; and 20 seeds silique^{-1}, or more for selfed pistils of SC plants. Comparing selfed (sl) with outcrossed (cr) seed, progeny classes with: i) sl/cr of 0.75 $\geq$ were defined as SC, ii) sl/cr of $\leq$ 0.25 were defined as SI, and iii) sl/cr of 0.25 > and < 0.75 was defined as pseudo-self-compatible (PSC). Observations typical of F_1 progeny are illustrated in Table 7.2. The sl/cr ratio was used as a standard for all further seed count comparisons involving SI and SC segregation.

Since the W and T lines had been crossed to CrGC-5 (a high erucic acid, high glucosinolate rapid cycling population developed by P. Williams; see

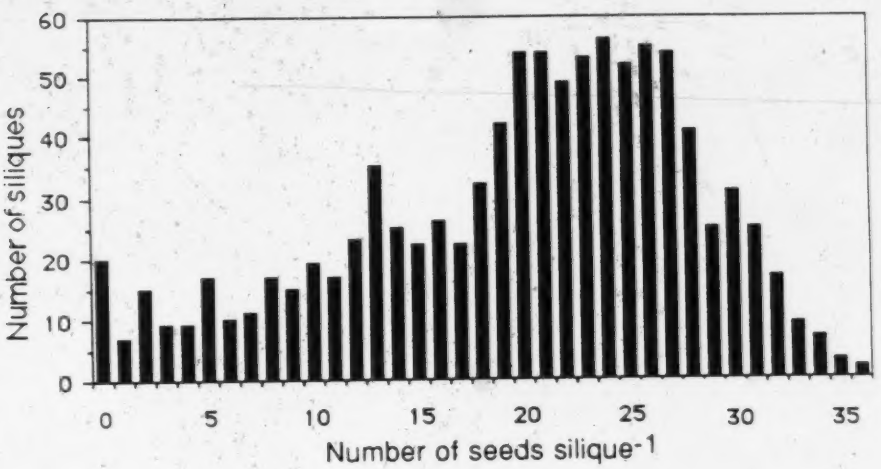


Figure 7.1 Number of seeds silique^{-1} in outcrossed T and W *B. napus* lines.

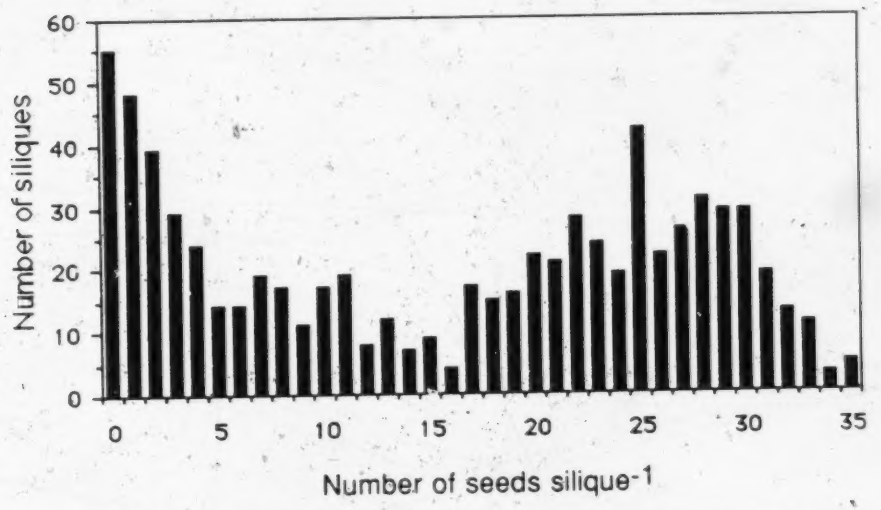


Figure 7.2 Number of seeds silique^{-1} in self-pollinated T and W *B. napus* lines.

Plant[1]	Self-pollinated (A)										Total	Cross-pollinated (B)[2]										Total	A/B	Class
T7-1	19	24	21	21	28	23	27	26	28	27	244	29	23	20	22	27	19	31	25	30	21	247	0.98	SC
T7-10	15	27	19	7	3	11	5	1	10	12	110	28	23	27	24	21	29	28	31	20	23	254	0.43	PSC
T7-4	0	0	0	0	0	0	0	0	0	0	0	20	12	21	23	30	20	25	32	20	25	228	0.00	SI
T7-14	0	0	0	0	0	0	0	0	0	0	0	0	0	16	6	3	12	0	11	9	14	71	0.00	SI

Table 7.2 Sporophytic compatible, partial sporophytic compatible and sporophytic incompatible *B. napus* plants classified on the basis of seed set from selfed and outcrossed pistils.

1 - Plant number selected from a population of 20 SC/T7 plants.
2 - Pistils outcrossed using mixed pollen from Westar, Global, Midas, Topas, and 86-411-6.

Appendix A) by P. Banks, each line was highly heterogeneous for agronomic performance, including plant vigour and seed yield. In some cases, plants exhibited poor seed set when selfed or outcrossed. Although the seed set may have been low for both self- and cross-pollinated pistils, these plants were classified as SI, SC, and PSC using the sl/cr ratio (see Table 7.2 Plant T7-14).

Plants with PSC have a functional incompatibility system but seed set from self- or incompatible cross-pollination may range from 1% to 100% of a compatible outcross (Litzow and Ascher, 1983), hence PSC plants were pooled with SI plants for data analysis. Some authors have allocated more refined incompatibility scores based on seed set such as the 6 levels described by Gowers (1989), but for the purposes of rapid screening, the 3 levels described above are sufficient. As indicated in Table 7.3 the PSC class was infrequent, accounting for 4.08% of the 98 F_1 plants. Although these designations are artificial, they permit a quantitative interpretation of the data. In practical terms however, only the most robust plants with extreme SI expression were selected for continued breeding (e.g. Plant T7-4 would be selected in preference to T7-14 in Table 7.2). Using the sl/cr ratio, the prediction for 1:1 segregation of SC and SI was tested by Chi square analysis (Table 7.3). Again, the model that SI is dominant and segregating 1:1 for SC:SI at one locus is accepted.

For both May and September seeded SC and SI rape, each SI plant was used as the pollen parent for bud crossing to the SC parent. Inasmuch as SI is not cytoplasmically determined the direction of the cross was a matter of convenience only. The procedure used was to remove a mature flower from the bagged SI line and cross three emasculated SC buds. The crossed SC buds were immediately bagged to prevent unwanted pollination. The procedure was repeated until all W and T plants were crossed with at least one of each of 86-411-6, Global, Midas, Topas, and Westar. Crosses which initially failed to

	Progeny Classes			Expected ratio (χ^2)
	SI	PSC[1]	SC	1:1
T7	10	1	8	0.5ns
T11	9	2	9	0.2ns
W3	8	0	12	0.8ns
W8	8	0	11	0.5ns
W13	1	0	11	0.2ns
Total	43	4	51	0.2ns

Table 7.3 Chi square analysis of F_1 T and W *B. napus* progeny.

1 - Partially sporophytic incompatible plants pooled with SI for analysis of expected ratios (see text for discussion).

produce seed were repeated. Since the T7, T11, W3, W8, and W13 parents from the May seeding proved to exhibit SI, September seeded SC/SI hybrids were not used further. Due to poor seed quality, not all combinations of F_1 SC/SI hybrids were selected for the proposed recurrent backcrossing to SC parents, and the seeding target of 20 seeds hybrid^{-1} was not met in some cases. This coupled with weak or absent SI forced deletion of some lines.

Selected F_1's and recurrent vparental SC lines were seeded September 3, 1987 in the greenhouse. To provide a uniform gene pool for succeeding backcrosses, parental SC lines were selfed and S_1 seed collected at maturity. The SC and SI plants were again identified by comparison of total seed counts from 10 selfed and 10 outcrossed pistils (sl/cr). Since selection of SI plants for the next generation of backcrosses was made after harvest, 3 pistils on a lateral branch of each F_1 plant were pollinated with the recurrent SC parent. Seed from the 3 siliques constituted the backcross (BC_1) seed source from selected F_1 plants. The frequency of F_1 SI and SC progeny classes is shown in Table 7.4. Pooled data was not significantly different from a 1 SI : 1 SC ratio when tested with χ^2 analysis ($0.25 < P < 0.50$).

F_1 hybrids:	Progeny classes SI	SC
86-411/T11	4	0
86-411/W13	-	-
Global/T7	6	6
Global/T11	5	1
Global/W8	7	6
Global/W13	4	3
Midas/T7	4	10
Midas/T11	9	10
Midas/W3	7	11
Midas/W8	6	12
Midas/W13	0	2
Topas/T7	9	8
Topas/T11	8	7
Topas/W3	9	10
Topas/W8	0	10
Topas/W13	0	9
Westar/W3	7	11
Westar/W8	-	-
Westar/W13	1	2
Westar/T11	5	10
Total	106	118

Table 7.4 *B. napus* lines segregating 1:1 for sporophytic incompatibility.

Segregation of dominant S alleles at one locus is consistent with the results obtained in all subsequent tests, and was stably inherited in all SC lines. If the S alleles in T and/or W were recessive (ss), none of the F_1's would have been expected to exhibit SI. Further, had any of the parents been homozygous dominant for an S allele (SS) in either T or W, all F_1's from that parent would have been expected to exhibit SI. The pooled data supports one allele pair at a single locus.

P1 Ss (SI) X ss (SC)

↓

F_1 1 Ss (SI) : 1 ss (SC)

Selected F_1 SI plants backcrossed to their recurrent SC parents gave progeny segregation ratios that were not significantly different from expected 1:1 ratios. SI reciprocal crosses made with SC parents [SC/SI crosses for the F_1; SC X (selected SI F_1) crosses for BC_1F_1] also segregated 1 SC : 1 SI thus ruling out cytoplasmic inheritance and genic male sterility since all segregants were fertile. Segregation ratios for bud selfed SI F_1 progeny (F_1S_1) were not significantly different from expected 3 SI : 1 SC ratios. No attempt was made to rule out gametophytic incompatibility (GI), although a weak GI response may have been present (Lewis *et al.*, 1988; Zuberi *et al.*, 1988). All data support dominance of one allele in an allele pair segregating 1:1 for SC and SI in both the T and W lines.

Since the usual order of crossing the terminal raceme was to first self then outcross 10 pistils, a test was made to determine if the number of seeds set for each silique remained constant over the first 30 flowers on the SC lines. If the seed set silique^{-1} did not remain constant over the first 30 flowers of the main raceme, then the cr/sf index would need to be appropriately adjusted. Seed set on the main raceme was tested on 1 86-411-6 plant, and 4 plants from each of Global, Midas, Topas, and Westar. Newly opened flowers on the main raceme from each of these SC plants were crossed with a pollen mixture from all SC parents. The seeds silique^{-1} for the first 30 siliques was counted at harvest for all SC plants tested. To test position effects, the observations for each raceme were tested as a single sample and analyzed using regression program S199 (developed by the Statistical Research Section, Engineering and Statistical Research Centre, Research Branch, Agriculture Canada, Ottawa) with the kind assistance of B. Thompson. If each plant is considered as one replicate, regression analysis shows that seed set silique^{-1} position effects are not consistent from replicate to replicate (Table 7.5). There is a tendency towards

fewer seeds silique^{-1} near the terminus of the raceme but the regressions are significant in only 8 of the 17 samples while the rest show essentially a flat plot over the 30 siliques. Even where the regression is significant, some samples, such as Westar-4, showed a significant decrease in seed set in only the last few siliques. On the basis of these results, pooling the 17 samples to obtain a single regression appeared to be unjustified. The analysis suggests a weak trend towards fewer seed near the terminus. Grand means for seeds set silique^{-1} for the first 10 siliques measured from the base of the main raceme, middle 10 siliques, and terminal 10 siliques are 24.9, 23.7, and 21.8 seeds silique^{-1} respectively. Based on the regression analysis (Table 7.5), no correction of sl/cr ratios appeared to be necessary. However, if progeny from SI crosses were assigned to classes taking into account the 12.4% decrease in seed set from the first and last 10 silique averages above, then an increase in plants recorded as SI would result. For example, Table 7.3 shows 43 SI : 4 PSC : 51 SC plants based on seed counts where sl/cr ratios of $0.25 \leq$ SI, $0.25 >$ PSC < 0.75, and SC ≥ 0.75. By increasing outcrossed seed set (flowers located at the terminal end of the raceme) by 12.4% before calculating the sl/cr ratio, the progeny classes change to 45 SI : 5 PSC : 48 SC, which remains insignificantly different from a 1:1 ratio when PSC progeny are pooled with SI progeny ($.975 < P < .990$).

Twenty seeds each from selected BC_1F_1's were planted July 6, 1988. Determination of SI was again by comparison of seed set between selfed and outcrossed pistils and each BC_1F_1 plant was backcrossed to the appropriate S_1 SC parent. The evaluation technique differed from the earlier test in that, for comparing seed set, 5 pistils were selfed (rather than the 10 pistils used previously) and 5 pistils were outcrossed by bud pollination using the recurrent pollen source from the S_1 parents. Outcrossed siliques were harvested as both

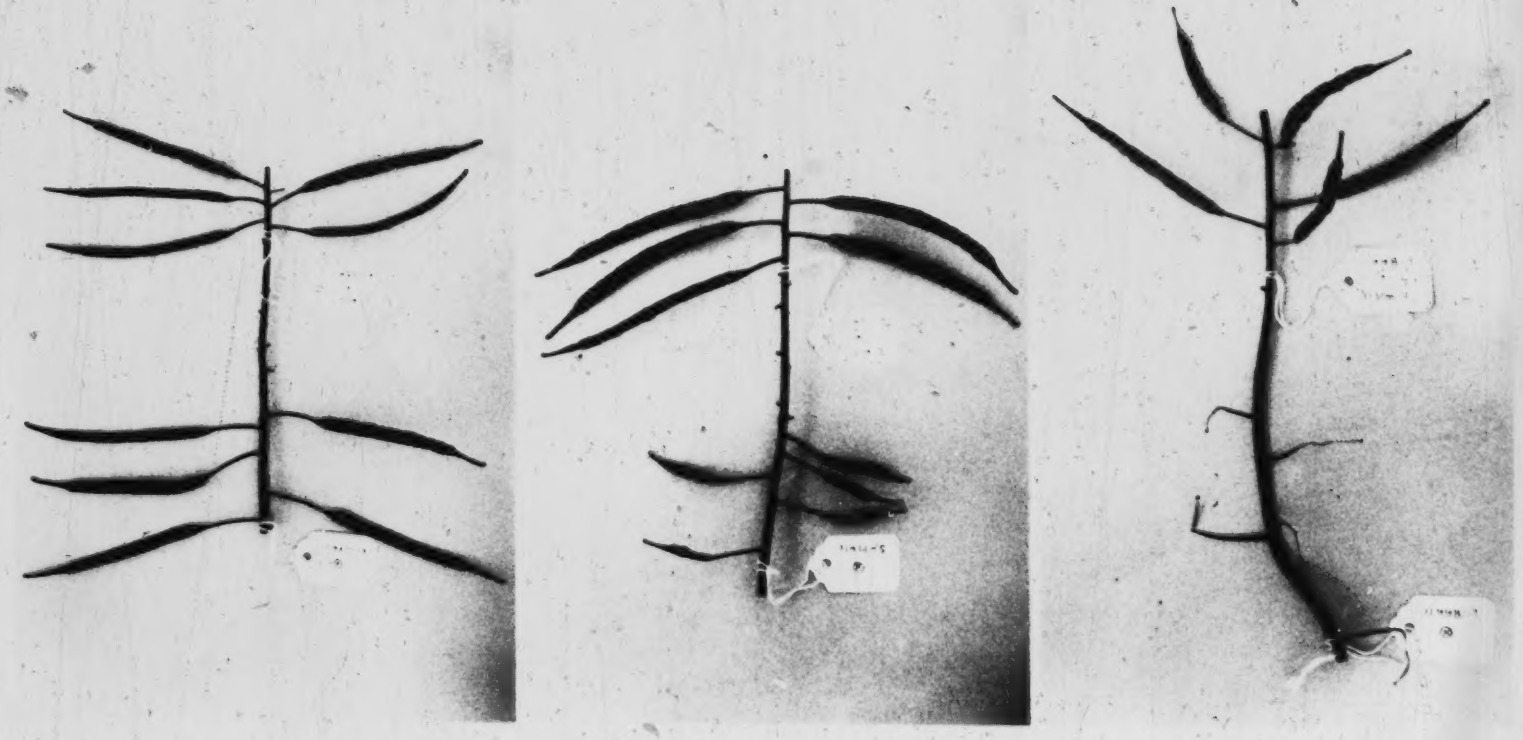
Lines	Slope	S.E.
86-411-6-1	-0.265	0.082 *
Global-1	0.054	0.046
Global-2	-0.073	0.040
Global-3	-0.023	0.076
Global-4	-0.164	0.047 *
Midas-1	-0.109	0.046 *
Midas-2	-0.602	0.106 *
Midas-3	0.036	0.056
Midas-4	0.039	0.041
Topas-1	-0.149	0.062 *
Topas-2	-0.011	0.043
Topas-3	0.027	0.047
Topas-4	0.026	0.049
Westar-1	0.067	0.050
Westar-2	-0.630	0.110 *
Westar-3	-0.329	0.128 *
Westar-4	-0.245	0.089 *

Table 7.5 Regression analysis of seeds silique^{-1} on the main raceme of sporophytic compatible *B. napus*.

the BC_2F_1 seed source and also used for comparison with the selfed seed to determine whether the plant was SI or SC. Selfing and outcrossing 5 pistils each was quite adequate to distinguish between SI and SC plants (Plate 7.1)

It may be argued that cross pollination preceded by emasculation decreases the number of viable seed set compared to selfing open flowers. If severe this could result in erroneous rejection of true SI plants. To determine if the comparison is valid, a number of flowers were selfed and outcrossed on S_1 Westar plants. Crosses were made randomly on terminal and lateral branches of each plant. Prior to self-pollination using open flowers, racemes were covered with glassine bags to prevent unwanted pollination. Selfed open flowers and selfed emasculated flowers were bagged immediately after crossing. Outcrossed open flowers and outcrossed emasculated flowers were

Plate 7.1 Terminal racemes of *B. napus* illustrating the effects of self-pollination (lower 5 pistils on each raceme) and cross-pollination (upper 5 pistils) in (A) sporophytic compatible, (B) pseudo-self-compatibility, and (C) sporophytic incompatible genotypes. Note that the cross-pollinated pistils result in siliques filled with seed in all cases, while self-pollination resulted in normal seed set for the sporophytic compatible plant, decreased seed set for the pseudo-self-compatible plant, and no seed set for the sporophytic incompatible plant.



A B C

crossed with mixed pollen from 86-411-6, Global, Midas, Topas, and Westar. Comparisons of the 4 crossing techniques is shown in Table 7.6.

Chi square analysis of selfed and outcrossed versus open and emasculated flowers using a 2 X 2 contingency table was not significant [$\chi^2_{(0.05,\ df\ 1)} = 3.841$. $\chi^2_{(calc)} = 0.73$]. The null hypothesis that seed set silique^{-1} is independent of self- or cross-pollination of open or emasculated flowers (Table 7.6) is thus accepted.

In order to more expeditiously screen for SC and SI plants, BC_1F_1 plants segregating for SI were assayed for pollen tube penetration into stigma papillae using fluorescent microscopy developed by Martin (1959). One lateral raceme was bagged just prior to flowering. When the raceme began to flower, a mature flower was removed and pollen

	Total No. seeds (A)	No. pistils crossed (B)	(A/B)
1. Selfed open flowers	1941	64	30.3
2. Outcrossed open flowers	1788	60	29.8
3. Selfed emasculated flowers	1597	54	29.6
4.Outcrossed emasculated flowers	1694	56	30.3
Total	7020	234	30.0

Table 7.6 Comparison of seed set for selfed and outcrossed flowers both prior to, and following anthesis on S_1 Westar plants.

transferred to newly opened flowers on the same raceme by rubbing the removed flower's dehiscing anthers against the pistils until a pollen layer was evenly spread across the stigma surface of at least 4 pistils. Pistils were again covered with glassine bags to inhibit cross contamination. Twelve to 24 hours after pollination, 2 to 4 stigmas from the self-pollinated flowers were removed

and fixed in a mixture of 80% ethanol, 37% formalin, and glacial acetic acid (8:1:1) for 5 hours, softened in 8.5 N sodium hydroxide for 12 hours, washed and stained in decolourized aniline blue dye (1.00 g aniline blue dye plus 7.07 g K3PO4 mixed in 1000 cc water adjusted to pH 7.0 using H2SO4) for 5 hours (modified from Crehu, 1968) and observed by means of fluorescent microscopy using a Wild model M20 microscope fitted with UG 1 ultra violet filters giving excitation wavelenths of 350-380 μm). The callose of pollen tubes fluoresces bright yellow to yellow-green. Self pollen from SI plants rarely penetrate the stigmatic papillae but if they do, tubes are arrested shortly after penetration. On the other hand, self-pollen from SC penetrate the stigma and grow down the style. The distinction between SI and SC plants was made on the basis of the number of pollen grains germinating with pollen tubes penetrating stigmatic papillae. Selfed stigmas showing 100 or more pollen tube penetrations were classified as SC. Those with 15 or less were classified as SI, thus adopting the SC and SI parameters used by Banks (1988). Banks selected 15 pollen tubes or less to depict sporophytic incompatibility since there were approximately 20 seeds silique^{-1} in the rapeseed cultivars used thus full seed set would not be possible. This compares favorably with the 20.8 seeds silique^{-1} average noted for the T and W lines (Figure 7.1). Pistils exhibiting intermediate pollen tube penetration, between 15 and 100, were classified as PSC and accounted for less than 10% of all observations. Since the objective was to rapidly screen for SI plants, no attempt was made to differentiate multiple strengths of incompatibility using, for example, the 8 tube penetration levels recommended by Wallace (1979). When UV analysis was compared with seed set data for the segregating BC_1F_1 population using the sl/cr ratios, all SI plants and SC plants were correctly identified. However, some plants designated as PSC using UV analysis ranged in seed set approaching both the SI and SC extremes. This

was considered inconsequential since only the strongest SI responses were selected for further breeding while PSC and SC plants were discarded. Since the acceptance or rejection of SI, SC and PSC plants using UV screening was correlated with seed set, tests were conducted to verify that this correlation was accurate.

Banks (1988) found a highly significant correlation between pollen tube count and seed set ($r = 0.62^{**}$) for the 9 SI lines he studied. To test this correlation in the T and W lines in the present study, 4 SI and 4 SC plants were isolated by means of UV analysis from BC_2F_1 plants derived from T and W lines segregating for SI and SC (BC_2F_1 plants were selected from an October 13, 1988 greenhouse planting of lines exhibiting SI and targeted for seed increase as discussed in a later section). The 4 SI plants consisted of 2 T plants (Global*3/T11 and Midas*3/T11) and 2 W plants (Global*3/W13 and Midas*3/W8); the 4 SC plants were derived from the same segregating populations as the corresponding SI plants. To first establish if removing the stigma from the pistil after pollination affected seed set, 20 pistils were self-pollinated on the terminal inflorescence at a rate of 4 pistils day^{-1} on 5 consecutive days. Twenty-four hours after each pollination, stigmas were paired and one of stigma from each pair was randomly excised using small scissors. At maturity the number of seeds $silique^{-1}$ were recorded. Data was analyzed as a 10 replicate 2x4x2 split-split plot where the factors were: SI or SC lines (2), 4 T and W genotypes (4), and removal or non-removal of stigma (2). The ANOVA and Tables of Means are presented in Table 7.7 and Table 7.8 respectively.

Source of variation	DF	SS	SS%	MS	VR
SCSI.Genotype.Cut					
.*Units* Stratum					
Block	9	26.056	0.11	2.895ns[1]	0.521
SCSI	1	23498.256	96.22	23498.256***	4228.312
Genotype	3	32.019	0.13	10.673ns	1.921
Cut	1	0.756	0.00	0.756ns	0.136
SCSI.Genotype	3	100.119	0.41	33.373***	6.005
SCSI.Cut	1	0.306	0.00	0.306ns	0.055
Genotype.Cut	3	6.519	0.03	2.173ns	0.391
	3	7.969	0.03	2.656ns	0.478
SCSI.Genotype.Cut					
Residual	135	750.244	3.07	5.557	
Total	159	24422.246	100.00	153.599	

Table 7.7 ANOVA table of seed set for sporophytic compatible and sporophytic incompatible *B. napus* with excised and non-excised stigmas following self-pollination.

1 - ns, and *** indicate not significant and significant at $P < 0.001$ respectively.

Grand Mean 12.93

Block	1	2	3	4	5	6	7	8	9	10
	12.56	12.44	12.94	13.63	13.19	12.44	12.88	13.13	13.50	12.63

SCSI	Cut	Uncut
	0.81	25.05

Genotype	Global*3/T11	Midas*3/T11	Global*3/W13	Midas*3/W8
	12.55	13.42	13.32	12.42

Cut	Cut	Uncut
	12.86	13.00

Genotype	Global*3/T11	Midas*3/T11	Global*3/W13	Midas*3/W8
SCSI				
SI	1.20	1.95	0.00	0.10
SC	23.90	24.90	26.65	24.75

Cut	Cut	Genotype
SCSI		
SI	0.70	0.93
SC	25.02	25.07

Cut	Cut	Uncut
Genotype		
Global*3/T11	12.35	12.75
Midas*3/T11	13.70	13.15
Global*3/W13	13.10	13.55
Midas*3/W8	12.30	12.55

Genotype	Global*3/T11		Midas*3/T11		Global*3/W13		Midas*3/W8	
Cut	Cut	Uncut	Cut	Uncut	Cut	Uncut	Cut	Uncut
SCSI								
SI	0.70	1.70	2.00	1.90	0.00	0.00	0.10	0.10
SC	24.00	23.80	25.40	24.40	26.20	27.10	24.50	25.00

Standard errors of differences of means:

Table	Block	SCSI	Genotype	Cut	SCSI Genotype	SCSI Cut	Genotype Cut	SCSI Genotype Cut
Rep	16	80	40	80	20	40	20	10
SED	0.833	0.373	0.527	0.373	0.745	0.527	0.745	1.054

Table 7.8 Mean seed set values for sporophytic compatible and sporophytic incompatible T and W *B. napus* lines with excised and intact stigmas.

The analysis indicates that removal or non-removal of stigmata 24 hours after pollination does not significantly affect seed set (P = 0.713), confirming the findings of Banks (1988). The removal or non-removal of stigmata was also not significantly affected by interactions between SC or SI plants (P = 0.815); by the 4 plant genotypes used (P = 0.760); or by the interaction of SI and SC genotypes (P = 0.698). Seed set for SI and SC plants were significantly different (< 0.001) as expected, and the interaction between genotypes and SI and SC plants was highly significant (< 0.001). The latter observation is interesting (note low seed set for SI Global*3/W13 and Midas*3/W8 compared to the two T derived SI plants, Table 7.8) since differences between genotypes and seed set was noted for all developed SI lines. Although the relative strengths of T and W derived SI lines were not measured, there were obvious differences in performance based on seed set and UV analysis. The W lines generally exhibited a stronger SI response in all backgrounds than did the T lines. Global and Midas backgrounds generally displayed the strongest SI responses while 86-411 and Westar showed a weaker response with Topas intermediate to the two groups. Inasmuch as the physiology and biochemistry of sporophytic incompatibility is poorly understood, it is not possible to speculate on interactions between the two SI alleles and different backgrounds. It is noteworthy, however, that the SC sources are from divergent backgrounds which undoubtedly accounts for the different 'strengths' of SI observed.

The same 8 plants were used to determine the correlation between pollen tube counts and seed set (this experiment was commenced on the assumption that the previous test would show that seed set is not effected by stigma removal). The procedure used was to self-pollinate 5 pistils on a lateral inflorescence on 2 consecutive days for each plant. Twenty four hours after each self-pollination, the stigmas were excised and pollen tube penetrations

determined by UV analysis. Siliques were harvested at maturity, and seed set data was collected for each corresponding pistil. The 4 self-pollinated SC plants (Global*3/T11, Global*3/W13, Midas*3/T11, and Midas*3/W8) all showed more than 100 pollen tube penetrations pistil^{-1} and seeds silique^{-1} ranged from 6 to 32 with only 2 siliques yielding less than 20 seeds. Seed set and pollen tube counts for the SI plants tested are shown in Table 7.9.

	Stigma Number:									
	1	2	3	4	5	6	7	8	9	10
Global*3/T11										
No. pollen tube penetrations pistil^{-1}	0	3	3	2	6	0	1	3	1	2
Number of seeds silique^{-1}	0	2	1	0	1	0	0	1	0	0
Midas*3/T11										
No. pollen tube penetrations pistil^{-1}	6	4	4	7	2	3	6	8	6	2
Number of seeds silique^{-1}	5	4	5	5	1	0	5	1	1	2
Global*3/W13										
No. pollen tube penetrations pistil^{-1}	1	0	3	2	2	1	0	0	1	0
Number of seeds silique^{-1}	0	0	0	0	0	0	0	0	0	0
Midas*3/W8										
No. pollen tube penetrations pistil^{-1}	0	1	0	0	0	0	3	1	3	4
Number of seeds silique^{-1}	0	0	0	0	0	0	0	0	0	0

Table 7.9 Relationship between seed set and pollen tube penetration in stigmata of sporophytic incompatible *B. napus*.

The Pearson product-moment correlation coefficient between seed set and pollen tube counts for SI plants (Table 7.9) is highly significant ($r = 0.66^{***}$) and compared favorably with Banks (1988) observations ($r = 0.62^{**}$). This analysis supports UV as a credible mechanism for rapid screening of SI and SC lines. Although pollen tube screening is considered a reliable method for studying SI (Zuberi *et al.*, 1981) seed set is the best test for some material (Gowers, 1989) since numerous irregularities in the sexual reproductive system, not mediated by sporophytic incompatibility, could lead to low seed set.

Hitherto S-genes in T and W have been treated as alleles at the same locus. To establish the allelic relationship between these sources, the 4 SI selections above were crossed in a matrix. If the alleles from the T and W sources are not the same, then crosses between plants would be expected to exhibit normal seed set. However, if the alleles are identical, then no seed set would be expected. To test the SI relationship, 20 buds from each of the SI plants Global*3/T11, Midas*3/T11, Global*3/W13, and Midas*3/W8 were emasculated at the bud stage (just prior to flowering), bagged, and allowed to mature to the growth stage normally encountered at first flowering. Test plants were then crossed in diallel fashion. Ten pistils were removed 24 hours after crossing for UV analysis and 10 left for latter seed set confirmation of UV analysis (Table 7.10)

Mean number of seeds silique^{-1}

	Male			
Female	(1)[1]	(2)	(3)	(4)
(1)	0.2	0.9	26.0	25.8
(2)	0.3	0.4	26.9	24.3
(3)	27.0	26.2	0.2	0.6
(4)	26.4	27.6	0.7	0.2

Mean number of pollen tubes stigma^{-1}

	Male			
Female	(1)	(2)	(3)	(4)
(1)	2.1	1.3	+	+
(2)	1.7	5.6	+	+
(3)	+[2]	+	1.0	1.0
(4)	+	+	1.8	1.4

Table 7.10 Seed set (A) and pollen tube (B) counts for a diallel cross of 4 SI plants introgressed with T and W alleles.

1 - (1) Global*3/T11; (2) Midas*3/T11; (3) Global*3/W13; (4) Midas*3/W8.
2 - Indicates 100 or more pollen tubes.

The diallel crosses for both seed set and pollen tube analysis convincingly demonstrate different S-alleles are present in the T and W sources. For example, the grand mean for seed set data (Table 7.10 A) T x T, T x W, W x T, and W x W is 0.4, 25.8, 26.8, and 0.4 seeds silique^{-1} respectively. The null hypothesis (Ho: 1:1:1:1) is clearly rejected (χ^2 $P << .001$).

7.1.1 Seed increase of SI and SC lines.

Nine single BC_1F_1 SI plants were selected for increase representing 9 of the possible 10 recurrent parent/T or W combinations. The criteria for selection was that each plant must exhibit strong sporophytic incompatibility as defined by the most extreme sl/cr and/or pollen tube data (Table 7.11).

T allele	W allele
411-6*3/T11	discontinued at BC_1F_1
Westar*3/T11	Westar*3/W3
Topas*3/T11	Topas*3/W3
Global*3/T11	Global*3/W13
Midas*3/T11	Midas*3/W8

Table 7.11 Selected sporophytic incompatible *B. napus* for greenhouse increase prior to field testing.

Forty seeds of each selected SI BC_1F_1 plant were sown October 13, 1988 in the greenhouse. Fluorescent microscopy was used to determine SI and SC plants. Increase of SC seed could be easily accomplished, but a method of increasing SI seed without cross contaminating lines posed a problem. The mechanism chosen for seed increase was to pollinate each line in isolation with bees.

To facilitate this experiment, eighteen isolation chambers were constructed to accommodate and separate the SI and SC plants. Each isolation chamber frame was constructed with the dimensions 1.2 m square x 2.1 m height. All sides were covered with 6 mill clear polyethylene plastic except for the 1.2 m x 2.1 m front panel which was covered by standard steel screening rated at 16 x 16 holes per inch^2. Each chamber was lighted by a single GTE Sylvania

Lucalux LU400 high pressure sodium lamp and the pots were held in a lined 1.0 m x 1.5 m plywood box. At flowering, each plant was tested for SI using UV fluorescent microscopy. Plants exhibiting PSC were retested for confirmation, and discarded if not clearly SC or SI. Each SC or SI plant was then placed in the appropriate isolation chamber. In each SI chamber, 1 SC plant was included for each 4 SI plants from the segregating population to provide a pollen source for SI seed increase.

Cross pollination of plants within each isolated chamber was attempted by using alfalfa leafcutting bees (*Megachile rotunda* Fab.) kindly provided by J. Lieverse (Agriculture Canada, Beaverlodge). On a weekly basis beginning October 21, 1988, 18 populations of leafcutter bees (each consisting of 40 ± 1 g of cells containing bee larvae) were placed in 20 x 100 mm petri dishes and incubated at 29 °C with a relative humidity of 50%. Hatching began approximately 30 days after initiation of incubation with an average of nine leafcutter bees g^{-1} emerging. At flowering, leafcutter bees were released within each isolation chamber on a weekly basis.

It became quickly evident that bee visitation to rape flowers was low under the conditions provided. When measuring the temperature gradient in the chamber, a full 10 °C difference separated the lower (15 °C) and upper portion (25 °C) of the isolation chamber. Also, many of the bees flew directly to the high pressure sodium lamp which was emitting a maximum base temperature of 210 °C. These bees became disoriented and/or burned then fell to the base of the chamber and never recovered. A number of modifications were attempted to ameliorate the unproductive conditions which included: adding a fan; screening the light to prevent bee contact; providing a ledge with a 200 watt incandescent light and a 30.5 x 56 cm plywood box containing wood nesting tunnels (each tunnel being 0.64 cm in diameter x 10.0 cm in length); providing a sugar water

food source for each chamber; and replacing the leaf cutter bees with honey bees (*Apis mellifera* L.) courtesy of D. Nelson (Agriculture Canada Research Station, Beaverlodge). None of the modifications lead to a significant increase in pollination activity. Besides the difficulty in maintaining a consistent heat gradient within each chamber, another possible contributor to low bee activity, which was not possible to change at the time, was spectral energy distribution of the 400-watt Lumalux lamp which was primarily active in the 550 to 650 nanometer (yellow and orange) wavelengths and had no significant energy in the ultraviolet regions. Thus natural conditions could not be emulated.

The bee *faux pas* was discarded when it became obvious that suitable seed set would not be achieved without manual intervention. This was accomplished using a small paint brush to cross pollinate all flowers within each chamber. One brush was assigned to each chamber thereby removing the threat of accidental cross pollination between chambers. In retrospect, increase of the SI lines would have been better accomplished using a sporophytic incompatibility inhibitor such as CO_2 or NaCl. When pollination was completed, the pollen source SC plants in the SI chambers was discarded. At maturity, each chamber was bulk harvested, dried on forced air racks, hand threshed and cleaned using a Dakota blower. A summary of the breeding procedure for a typical derivation is shown in Figure 7.3.

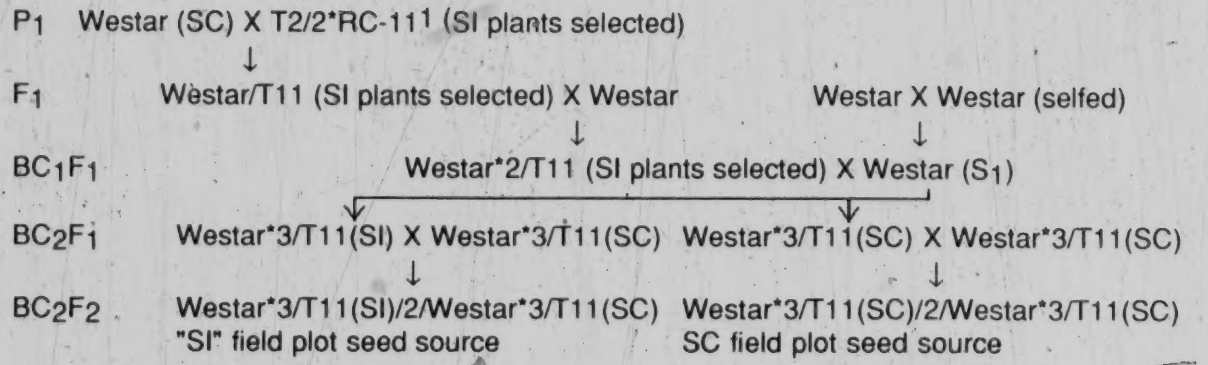

P1 Westar (SC) X T2/2*RC-11[1] (SI plants selected)
F1 Westar/T11 (SI plants selected) X Westar
Westar X Westar (selfed)
BC1F1 Westar*2/T11 (SI plants selected) X Westar (S1)
BC2F1 Westar*3/T11(SI) X Westar*3/T11(SC)
Westar*3/T11(SC) X Westar*3/T11(SC)
BC2F2 Westar*3/T11(SI)/2/Westar*3/T11(SC)
"SI" field plot seed source
Westar*3/T11(SC)/2/Westar*3/T11(SC)
SC field plot seed source

Figure 7.3 Pedigree of sporophytic incompatibility introgression into *B. napus* and seed increase.

1 - Sporophytic incompatible T11 selection from P. Banks' T SI line.

7.1.2 Field evaluation of sporophytic compatible and sporophytic incompatible lines.

Development of true isogenic lines would take up to 6 back crosses (Allard, 1960) to the recurrent parent. Time constraints prohibited more than 2 backcrosses for the T and W lines, but this limitation does not invalidate a comparison between SI and SC lines to establish whether or not possessing active sporophytic incompatibility is detrimental to agronomic performance. Some CMS A-lines, for example, are unstable in different environments, less attractive to bee forage, and agronomically less vigorous than their B- or R-line counterparts. The following field trials were designed to determine whether or not a similar 'penalty' existed for lines containing sporophytic incompatibility alleles.

Sufficient BC_2F_2 seed was produced in the greenhouse trial to permit field trials at three sites: Beaverlodge Site A (55° 12' N, 119° 25' W; Landry-Albright association soil type), Beaverlodge Site B (55° 11' N, 119° 22' W; Landry-Albright association) and Edmonton (53° 30' N, 113° 31' W; Black Chernozems soil). In Edmonton and Beaverlodge Site A, SC and SI seed from the 9 lines was seeded in the summer of 1989 in four replications of a 9 x 2 split plot design where the main plots were the 9 parents, and the sub-plots the SC and SI lines. Note that since each BC_2F_1 SI plant was crossed with SC rape to establish a seed source, each "SI" line consists of a 1:1 ratio of SI and SC BC_2F_2 plants. The split-plot design was selected since the interaction of interest was to determine how the SI and SC material from each parent performed. The split-plot design partitions the error term into two. Compared to randomized complete block experiments, the split-plot design increases the precision for the sub-plot treatments and interaction at the expense of precision for the main-plot treatments (LeClerg *et al.*, 1966). The Beaverlodge Site B test

was set up in the same manner except that parents 411-6*3/T11, Global*3/T11, Midas*3/T11, and Topas*3/T11 were omitted due to insufficient seed.

Seeds were planted at a depth of 2.5 cm at all sites; planting dates were May 9, 1989 for both Beaverlodge Site A and Edmonton while Beaverlodge Site B was planted on May 31, 1989. In Beaverlodge, each sub-plot consisted of 6 rows 8 m in length seeded at 0.9 g seed row^{-1} and bordered by 2 rows of the spring wheat *Triticum aestivum* L. var. Neepawa seeded at 15 g row^{-1}. Mono-ammonium phosphate (11-51-0) was drilled with the seed at 67 kg ha^{-1}; the pesticides Furadan-10 granular (systemic insecticide containing 10% carbofuran active ingredient) and Benolin-R (insecticide and fungicide dust contain 6% benomyl + 10% thiram + 50% lindane active ingredients) were mixed with seed packages for each row at a rate of 20 g Furadan 10G (2.7 kg/ha) and 0.30 g Benolin-R/0.9 g seed package. Each rape sub-plot (excluding wheat borders) was 1.2 x 8 m in size with wheat and rape row spacing at 20 cm. Prior to harvest, each 8 m row was trimmed back 1 m on either side giving a final rape plot size of 1.2 x 6 m.

In Edmonton, each sub-plot consisted of 4 rows 6 m in length with 23 cm spacing between rows and 23 cm between plots. The seeding rate and pesticide application for each row was the same as that used at Beaverlodge but no wheat boarders were seeded at the Edmonton location. Mono-ammonium phosphate (11-55-0) was broadcast prior to seeding at 56 kg ha^{-1}.

Agronomic parameters evaluated included days to flower, days to end of flowering, plant height, lodging, date of physiological maturity, number of siliques on the terminal raceme, number of seeds per terminal raceme, number of seeds $silique^{-1}$ on the terminal raceme, mg $seed^{-1}$ on the terminal raceme, yield, mg $seed^{-1}$ per sub-plot, and percent oil content.

In Beaverlodge, date of flowering was recorded when 50% of the plants had at least one open flower. End of flowering was noted when 80% of the plants had completed flowering. For the Beaverlodge sites, data for all agronomic variables described was collected for Site A. At Site B, data was collected only for days to flower, and days to end of flowering since frost damage on September 9, 1989 prevented further meaningful analysis. Plant height was based on average measurements of extended terminal racemes from 10 randomly selected plants. Lodging values were estimated from the ratio of standing height to the plant height. Physiological maturity was recorded when 50% of the seeds had changed colour. Number of siliques per terminal raceme was determined by removing and individually counting the siliques on the terminal racemes from 10 consecutive mature plants 1 m into the third row of each trimmed plot. The siliques from these 10 racemes were dried in an oven for 48 hours at 35 °C, hand threshed, screened, blown in a Dakota blower and weighed (± 0.0001 g) using an analytical balance. From this bulk seed, 1000 ± 2 seeds were counted using a Model 850-2 electronic seed counter (The Old Mill Company, Savage, Maryland, 20763). The thousand seeds were weighted (± 0.0001 g to determine seed weight for terminal racemes from which number of seeds per terminal raceme and the number of seeds silique^{-1} on the terminal raceme were calculated. At physiological maturity, all plots were sprayed with the desiccant Reglone (200 g L^{-1} diquat active ingredient) at 3.5 L ha^{-1}. Ten days after spraying, all plots were harvested (September 29, 1989) by direct combining the 6 rows (7.2 m^2) of each sub-plot using a Hegge plot combine. Seed was then screened, placed in paper bags, dried to 7.0% moisture in hot air (30 °C) chambers, weighed and yields converted to kg ha^{-1} by multiplying plot yield by 1.367. Sub-samples were later redried. Seed weight per sub-plot was determined by electronic seed counting of 1000 seeds from a random

sample of the bulk sub-plot harvest. Oil content was determined (± 0.1%) by nuclear magnetic resonance (NMR) using a Newport Analyser MK III A (Oxford Instruments Group, England, MK14-5AW) calibrated with samples from which percent oil had previously been determined by the Swedish tube method.

At the Edmonton site, data was discarded for days to flower, days to end of flowering, and days to mature since the cooperator recorded data for replicate one only. With the exception of plot size and harvest technique in Edmonton, treatment of agronomic characters was the same for all 3 sites. In Edmonton, the center 4.35 m of the 2 center rows of each sub-plot was hand harvested (2.0 m^2), placed in cotton bags, dried, threshed, screened, and weighed. Conversion of plot yield to kg ha^{-1} was made by multiplying the yield by 4.998. One hundred gram sub-samples from sub-plots were sent to Beaverlodge for further analysis. Samples were redried for 48 hours at 35 °C in an oven, left at room temperature for two days, and screened using a Dakota blower before testing for percent oil content.

7.2 Results and Discussion

Statistical analysis were performed using release 4.04B of Genstat (Lawes Agriculture Trust, 1984). Analysis of variance for height, kg ha^{-1}, mg $seed^{-1}$ for the terminal raceme, plot mg $seed^{-1}$, and percent oil for Beaverlodge Site A and Edmonton is shown in Table 7.12. Data was transformed, using natural log transformation, for all parameters except percent oil. Very little experimental variation was attributable to the SC and SI genotypes as indicated by percent sum of squares (Table 7.12). No significant differences in performance were present between the SC and SI genotypes for any of the agronomic traits measured. Varietal interactions with SC and SI genotypes were significant for yield (kg ha^{-1}) and percent oil, but in both cases, superior performance was not

attributable exclusively to either SC or SI as indicated in the table of means (Table 7.13). Analysis of variance (using a Poisson distribution) and table of means for lodging, number of siliques on the terminal raceme, and seeds silique^{-1} for Beaverlodge Site A and Edmonton are presented in Table 7.14. No significant differences existed between the SC and SI genotypes or between varieties and the SCSI genotypes. The same is true for days to flower, days to end of flowering, and days to mature tested at Beaverlodge Site A and Site B (Tables 7.15 and 7.16). Varietal differences were highly significant for all agronomic traits studied confirming the parental germplasm used for SI introgression had diverse origins.

The data on agronomic traits compared in this study indicates that there exists no significant difference between lines with introgressed SI and similar lines. Hence, there appears to be no 'biological cost' associated with sporophytic incompatibility. Flower morphology and general physiology of SI and SC plants were indistinguishable in greenhouse and field conditions. This is crucial since the introgression of dominant S-allele(s) into rape generates the genetic equivalent of dioecy with obligate outcrossing to achieve seed set. With the development of allogamous rape from predominantly autogamous parents, the *de novo* plants, assuming they are entomophilous, require suitable nectar, olfactory, and floral characteristics to encourage insect animal vectors to maximize cross pollination for maximum F_1 seed set. Although the flower morphology and bee visitation was examined superficially in the field (and nectar production not observed), there appeared to be no preference between the SC and SI plots. Further, there was no significant difference in flowering time between near isogenic SC and SI lines, synchronization of flowering again being essential for hybrid seed development. In essence, the sporophytic system does not appear to be hampered by some of the phenological

Source of variation	d.f.	Percent Sum of Squares				
		Height (cm)	Kilograms hectare^{-1}	Terminal Raceme mg seed^{-1}	Plot mg seed^{-1}	Percent Oil
Location	1	0.77	48.53	5.89	1.68	66.34
Location.Block	6	14.71	1.51	2.00	2.31	0.61
Location.Block.Plot						
Variety	8	28.20***1	17.40***	62.60***	83.71***	26.72***
Residual	56	37.20	18.34	12.43	6,27	4.24
Total	64	65.39	35.74	75.03	89.98	30.96
Location.Block.Plot.Subplot						
SCSI	1	0.00ns	0.04ns	0.60ns	0.04ns	0.01ns
Variety.SCSI	8	1.95ns	3.37*	1.21ns	1.00ns	0.86***
Residual	63	17.18	10.82	15.27	4.99	1.21
Total	72	19.13	14.23	17.08	6.04	2.08
Grand Total	143	100.00	100.00	100.00	100.00	100.00
Grand Mean		4.83	7.91	1.21	1.20	44.47
Total number of observations	144					

Table 7.12 Analysis of variance for height, kilograms hectare^{-1}, terminal raceme mg seed^{-1}, plot mg seed^{-1}, and percent oil tested at Beaverlodge Site A and Edmonton.

1 - ns, *, and *** indicate not significant, significant at $P < 0.05$, and $P < 0.001$ respectively.

Means:	Height (cm)		Kilogram hectare^{-1}		Terminal Raceme mg seed^{-1}		Plot mg seed^{-1}		Percent Oil	
SCSI	SC	SI	SC	SI	SC	SI	SC	SI	SC	SI
	4.833	4.832	7.918	7.909	1.200	1.220	1.197	1.201	44.44	44.51
SCSI*Variety	SC	SI	SC	SI	SC	SI	SC	SI	SC	SI
Global_T	4.839	4.860	7.994	7.802	1.086	1.085	1.125	1.094	44.16	45.29
Global_W	4.885	4.894	7.979	8.018	1.111	1.101	1.114	1.119	45.14	45.18
Midas_T	4.835	4.826	7.732	7.831	1.233	1.268	1.214	1.227	43.66	43.56
Midas_W	4.794	4.776	7.876	7.909	1.284	1.315	1.248	1.250	43.21	43.63
Topaz_T	4.855	4.850	7.992	8.050	1.079	1.085	1.077	1.054	45.71	46.43
Topaz_W	4.830	4.852	7.998	7.946	1.118	1.150	1.115	1.124	46.45	46.01
Westar_T	4.843	4.818	7.952	8.003	1.281	1.308	1.250	1.277	44.63	44.71
Westar_W	4.776	4.788	8.059	7.920	1.357	1.337	1.331	1.331	46.24	45.28
86-411_T	4.840	4.826	7.685	7.701	1.252	1.329	1.299	1.336	40.80	40.50
SED for SCSI	0.006		0.028		0.012		0.006		0.09	
SED for Variety*SCSI	0.024		0.075		0.037		0.019		0.42	
SED for Means Variety*SCSI[1]	0.018		0.063		0.037		0.017		0.26	

Table 7.13 Table of means for height, kilograms hectare^{-1}, terminal raceme mg seed^{-1}, plot mg seed^{-1}, and percent oil tested at Beaverlodge Site A and Edmonton.

1- SED for means within the same level of Variety*SCSI.

		Lodging		Number of siliques on the terminal raceme		Number of seeds silique^{-1}	
	d.f.	Deviance		Deviance		Deviance	
Location.Block	7	4.628		305.339		8.592	
Variety	8	19.894**		22.568**		40.935***	
SCSI	1	0.041ns		1.947ns		3.392ns	
Variety.SCSI	8	0.385ns		2.735ns		1.652ns	
Residual	119	14.012		51.412		28.513	
Means: SCSI		SC	SI	SC	SI	SC	SI
		1.500 (0.144)[1]	1.542 (0.146)	27.39 (0.62)	28.62 (0.63)	22.10 (0.55)	20.68 (0.54)
	SCSI*Variety	SC	SI	SC	SI	SC	SI
	Global_T	1.625 (0.450)	1.500 (0.433)	26.24 (1.81)	27.61 (1.86)	23.75 (1.72)	21.78 (1.65)
	Global_W	1.250 (0.395)	1.250 (0.395)	29.24 (1.91)	31.85 (2.00)	24.19 (1.74)	22.15 (1.66)
	Midas_T	2.000 (0.500)	2.250 (0.530)	25.16 (1.77)	26.24 (1.81)	21.50 (1.64)	20.41 (1.60)
	Midas_W	2.625 (0.573)	2.500 (0.559)	26.63 (1.82)	26.24 (1.81)	19.80 (1.57)	19.56 (1.56)
	Topaz_T	1.125 (0.374)	1.125 (0.374)	28.74 (1.90)	31.04 (1.97)	23.67 (1.72)	21.43 (1.64)
	Topaz_W	1.000 (0.353)	1.000 (0.353)	28.24 (1.88)	31.93 (2.00)	22.89 (1.69)	21.04 (1.62)
	Westar_T	1.375 (0.414)	1.375 (0.414)	26.70 (1.83)	25.04 (1.77)	23.60 (1.72)	24.30 (1.74)
	Westar_W	1.125 (0.375)	1.375 (0.414)	25.20 (1.77)	26.59 (1.82)	22.98 (1.69)	20.98 (1.62)
	86-411-6_T	1.375 (0.414)	1.500 (0.433)	30.38 (1.95)	31.06 (1.97)	16.53 (1.44)	14.49 (1.35)

Table 7.14 Analysis of variance and table of means for lodging, number of siliques on the terminal raceme, and number of seeds silique^{-1} tested at Beaverlodge Site A and Edmonton.

1 - (SE). Note: Standard errors are approximate since the model is not linear.

		Percent Sum of Squares		
Source of variation	d.f.	Days to flower	Days to end of flowering	Days to mature
Block Stratum	3	3.75	1.96	2.95
Block.Plot Stratum				
Variety	8	58.79***	83.32***	84.58***
Residual	24	19.77	11.97	8.58
Total	32	78.56	95.29	93.16
Block.Plot.Subplot Stratum				
SCSI	1	0.25ns	0.22ns	0.05ns
Variety.SCSI	8	3.83ns	0.91ns	0.47ns
Residual	27	13.62	1.62	3.36
Total	36	17.69	2.75	3.89
Grand Total	71	100.00	100.00	100.00
Grand Mean		4.13	4.48	4.84
Total number of observations	72			

Means: SCSI	SC	SI	SC	SI	SC	SI
	4.130	4.128	4.476	4.478	4.842	4.842
SCSI*Variety	SC	SI	SC	SI	SC	SI
Global_T	4.147	4.154	4.491	4.500	4.856	4.854
Global_W	4.143	4.135	4.497	4.500	4.858	4.864
Midas_T	4.139	4.143	4.489	4.494	4.836	4.834
Midas_W	4.127	4.115	4.469	4.472	4.832	4.830
Topas_T	4.147	4.135	4.500	4.500	4.873	4.871
Topas_W	4.107	4.107	4.457	4.457	4.854	4.852
Westar_T	4.131	4.127	4.457	4.451	4.826	4.826
Westar_W	4.115	4.119	4.460	4.460	4.826	4.822
86-411_T	4.115	4.119	4.463	4.466	4.820	4.820
SED for SCSI	0.003		0.001		0.001	
SED for Variety*SCSI	0.009		0.006		0.006	
SED for means Variety*SCSI[1]	0.008		0.003		0.004	

Table 7.15 Analysis of variance and table of means for days to flower, days to end of flowering, and days to mature at Beaverlodge Site A.

1- SED for means within the same level of Variety*SCSI.

		Percent Sum of Squares	
		Days to flower	Days to end of flowering
Source of variation	d.f.		
Location Stratum	1	96.42	98.20
Location.Block Stratum	6	0.45	0.18
Location.Block.Plot Stratum			
Variety	4	1.22***	1.02***
Residual	28	1.36	0.51
Total	32	2.59	1.53
Location.Block.Plot.Subplot Stratum			
SCSI	1	0.02ns	0.00ns
Variety.SCSI	4	0.05ns	0.01ns
Residual	35	0.47	0.08
Total	40	0.54	0.09
Grand Total	79	100.00	100.00
Grand Mean		3.996	4.34
Total number of observations	80		

Means: SCSI	SC	SI	SC SI	
	3.994	3.990	4.340	4.340
SCSI*Variety	SC	SI	SC	SI
Global_W	4.015	4.003	4.366	4.367
Midas_W	3.978	3.982	4.333	4.334
Topas_W	3.976	3.978	4.333	4.335
Westar_T	4.014	4.007	4.333	4.332
Westar_W	3.985	3.979	4.338	4.332
SED for SCSI	0.003		0.001	
SED for Variety*SCSI	0.010		0.006	
SED for means Variety*SCSI[1]	0.007		0.003	

Table 7.16 Analysis of variance and table of means for days to flower, and days to end of flowering tested at Beaverlodge Site A and Site B.

1- SED for means within the same level of Variety*SCSI.

parameters and reduction of pollen forage vectors suffered by other pollen control mechanisms, notably the CMS system (see Literature Review for discussion).

8.0 Summary and Conclusion

Seven lines and 5 varieties of summer rape were crossed in diallel. Combining ability estimates were made for flowering, yield, mg seed-^{1}, and percent seed oil content. General, specific, and reciprocal combining abilities were significant for all traits supporting earlier reports of the superior performance of F_1 hybrids in oilseed rape (Schuster and Michael, 1976; Shiga, 1976; Campbell and Kondra, 1978; Morice, 1978; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985).

Interplant outcrossing rate was determined for 16 lines of summer rape using a biochemical marker, erucic acid, to establish self- or cross-pollination. Rates of outcrossing for each line were compared with previously established outcrossing rates for parents of each line using rank correlation tests. Depending on the correlation test used, ranks were marginally significant indicating that a genetic component may be responsible for the various outcrossing rates observed. The mean outcrossing rate (55.3%) for the one site tested is higher than hitherto reported in the literature.

Tests were conducted to see if partial or 'residual' sporophytic incompatibility (RSI) was present in domestic rape by reciprocally cross pollinating rape using mixed pollen from both parents, then screening progeny to determine outcrossing rates. Initial trials using erucic acid as the only marker were unsuccessful since it was not possible to clearly distinguish self- from outcrossed progeny from the high erucic female acid parent. This problem was corrected by producing a recessive pale-petal mutant/high erucic acid line and

reciprocally crossing individual plants with individual wild-type flower/low erucic acid plants using mixed pollen. Outcrossing rates were determined by either using erucic acid content or flower colour depending on the direction of the cross. One Westar plant was identified with potential RSI, and SI controls were successfully identified.

Introgression of SI into summer rape was attempted using SI *B. oleracea*, *B. campestris*, and *B. napus* ssp. *rapifera* (Swedes). Introgression was easily accomplished using the latter two species, but unsuccessful with *B. oleracea*. The relationship of 6 plants with introgressed Swede SI alleles was determined by reciprocally chain crossing progeny and screening using a pollen tube assay from which 4 alleles were identified. In addition, 47 rape lines with *B. campestris* backgrounds selected from an ongoing breeding program were screened for SI by observing seed set when self-pollinated. No SI plants were identified in these lines suggesting that SI is easily lost or selected against.

Two dominant SI alleles were introgressed into 5 lines of SC rape. Each line was backcrossed for two generations and SI plants selected by pollen tube or seed set assays. Segregating SI and SC lines were increased and grown in field conditions at three sites. No significant differences in performance were observed between SC and SI lines for any of the agronomic traits measured suggesting that there is no 'biological cost' associated with sporophytic incompatibility.

The hybrid potential in rape encourages the development of pollen control mechanisms to exploit heterosis. Sporophytic incompatibility as such a pollen control mechanism is currently used in the production of some *B. oleracea* vegetables, but has been largely ignored in oilseed rape until recently. Inbreeding depression makes the SI system difficult to maintain in *B. campestris*, but more commercially viable in *B. napus* where inbreeding

depression is moderate. Commercial production of hybrid *B. napus* oilseed rape has been hampered by the identification of suitable S-alleles in parents of high combining ability, and by the high costs associated with maintaining SI rape. Recently, the latter problem has been reduced by CO_2 and NaCl applications, although identification of favorable SI lines remains an ongoing breeding problem. However, the fact that hybrid seed can be collected from both parents; suitable SI parents exhibit near complete outcrossing, and the low biological cost associated with the system make sporophytic incompatibility an attractive candidate for future pollen control development.

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10.0 Appendices

Appendix A - Seed source.

1982_C5 (77-4397E). *B. napus* ssp. *oleifera*. Line with 50 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1982_C10 (77-4397E). *B. napus* ssp. *oleifera*. Line with 10 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_B2 (DI820). *B. napus* ssp. *oleifera*. Line with 15 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_B7 (DI820). *B. napus* ssp. *oleifera*. Line with 15 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_B8 (DI820). *B. napus* ssp. *oleifera*. Line with 55 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_B9 (DI820). *B. napus* ssp. *oleifera*. Line with 55 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_C1 (77-4397E). *B. napus* ssp. *oleifera*. Line with 5 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1983_C6 (77-4397E). *B. napus* ssp. *oleifera*. Line with 70 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_A4 (Westar). *B. napus* ssp. *oleifera*. Line with 7.7 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_A6 (Westar). *B. napus* ssp. *oleifera*. Line with 29.8 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_B1 (DI820). *B. napus* ssp. *oleifera*. Line with 2.1 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_B15 (DI820). *B. napus* ssp. *oleifera*. Line with 38.3 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_C14 (77-4397E). *B. napus* ssp. *oleifera*. Line with 2.1 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_C22 (77-4397E). *B. napus* ssp. *oleifera*. Line with 29.8 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_E3 (WW48-29-80). *B. napus* ssp. *oleifera*. Line with 6.4 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

1984_E8 (WW48-29-80). *B. napus* ssp. *oleifera*. Line with 28.3 % outcrossing. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Rakow.

77-1999B. *B. napus* ssp. *oleifera*. Canola line developed by: Department of Plant Science, University of Alberta, Edmonton, Alberta. T6G 2P5.

81-50864B. *B. napus* ssp. *oleifera*. Canola line developed by: Department of Plant Science, University of Alberta, Edmonton, Alberta. T6G 2P5.

86-411-6 (N85-5632). *B. napus* ssp. *oleifera*. Experimental line derived from a cross between R3529, a Chinese line, and Westar. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2.

Andor. *B. napus* ssp. *oleifera*. Canola cultivar developed by: Department of Plant Science, University of Alberta, Edmonton, Alberta. T6G 2P5.

DH12439. *B. napus* ssp. *oleifera*. Pale-yellow petalled rape derived from a double haploid. From: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Courtesy of G. Seguin-Swartz.

DI-820. *B. napus* ssp. *oleifera*. Canola line developed by: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2.

Early Snowball. *B. napus* ssp. *rapifera*. Swede from: Seed Center Ltd., 14610 127 St., Edmonton, Alberta.

Global. *B. napus* ssp. *oleifera*. Canola quality cultivar developed at Svalof AB, S-268 00 Svalof, Sweden. Certified seed used.

Hanna. *B. napus* ssp. *oleifera*. Canola line developed at Weibull AB, Box 520, S-261, 24 Landskrona, Sweden.

Hero (S82-4362). *B. napus* ssp. *oleifera*. High erucic acid rapeseed cultivar developed by: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba. R3T 2N7. Courtesy of P. McVetty.

Karat. *B. napus* ssp. *oleifera*. Canola line developed at Svalof AB, S-268 00 Svalof, Sweden.

Laurentian var. Fortune. *B. napus* ssp. *rapifera*. Swede No. 372C from: Stokes Seed Ltd., St. Catharines, Ontario. L2R 6R6.

Midas. *B. napus* ssp. *oleifera*. Low erucic acid rapeseed developed by Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2. Certified seed used.

PGR 4123. *B. oleracea*. From: The Plant Gene Resources of Canada, Ottawa Research Station, BLDG. 75. Research Branch, Agriculture Canada, Ottawa, Ontario. K1A 0C6. Courtesy of B. Fraleigh.

PGR 4130. *B. oleracea*. From: The Plant Gene Resources of Canada, Ottawa Research Station, BLDG. 75. Research Branch, Agriculture Canada, Ottawa, Ontario. K1A 0C6. Courtesy of B. Fraleigh.

Purple Top Laurentian. *B. napus* ssp. *rapifera*. Swede from: Seed Center Ltd., 14610 127 St., Edmonton, Alberta.

Purple Top White Globe var. Navet. *B. napus* ssp. *rapifera*. Swede from: Bowseed Alberta Nurseries, Bowden, Alberta, T0M 0K0.

Reston. *B. napus* ssp. *oleifera*. High erucic acid rapeseed cultivar developed by: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba. R3T 2N7. Certified seed used.

S76-3048. *B. napus* ssp. *oleifera*. Canola line developed by: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba. R3T 2N7.

S76-3513. *B. napus* ssp. *oleifera*. Canola line developed by: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba. R3T 2N7.

SV02231. *B. napus* ssp. *oleifera*. Canola line developed at Svalof AB, S-268 00 Svalof, Sweden.

T2/2*RC *B. napus* ssp. *oleifera*.
Pedigree: F3 of RC[1] x (RC x BC_4F_4 of Z[2] x Topas[3])
1 - RC is P. Williams rapid cycling population.
2 - Z was derived from B. napus.
3 - Recurrent parent.
The sporophytic incompatible Z allele was obtained from the Scottish Crops Research Institute, Invergowrie, Dundee DD2 5DA, Scotland. A line homozygous for the allele was crossed and backcrossed to CrGC-5 a high erucic acid, high glucosinolate rapid cycling population developed by P. Williams, Cruciferae Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison WI 53706, USA. Seed released courtesy of P. Banks, Crop Science Department, OAC, University of Guelph and the Ministry of Agriculture and Food.

Tobin. *B. campestris* ssp. *oleifera*. Canola quality spring Turnip rape cultivar developed by Agriculture Canada, Saskatoon, Saskatchewan, Canada. S7N 0X2. Certified seed used.

Topas. *B. napus* ssp. *oleifera*. Canola quality cultivar developed at Svalof AB, S-268 00 Svalof, Sweden. Certified seed used.

W1/2*RC. *B. napus* ssp. *oleifera*.
Pedigree: F3 of RC[1] x (RC x BC_4F_4 of B33-1[2] x Westar[3])
1 - RC is P. Williams rapid cycling population.
2 - B33-1 was derived from cv. Candle.
3 - Recurrent parent.
The sporophytic incompatible allele was introgressed into Brassica napus from the B. campestris canola cultivar Candle. A line homozygous for the allele was crossed and backcrossed to CrGC-5 a high erucic acid, high glucosinolate rapid cycling population developed by P. Williams, Cruciferae Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison WI 53706, USA. Seed was released courtesy of P. Banks, Crop Science Department, OAC, University of Guelph and the Ministry of Agriculture and Food.

Westar. *B. napus* ssp. *oleifera*. Canola quality spring B. napus cultivar developed by Agriculture Canada, Saskatoon, Saskatchewan, Canada. S7N 0X2. Certified seed used.

ZN7-558. *B. napus* ssp. *oleifera*. Canola line developed by: Agriculture Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2.

Appendix B - Potting mixture (modified from a mixture developed by W. Leonard, Agriculture Canada Research Station, Saskatchewan, Saskatoon).

Materials:

1. 1 standard size pressure packed bag of sphagnum peat moss ($113\ dm^2 = 4\ ft^3$).
2. 2 bags of horticulture grade vermiculite (7 kg each) or 3 bags of standard (5 kg) vermiculite (brand name: terra-lite).
3. 3 kg osmocoat (14-14-14) controlled release fertilizer.
   Nitrogen: 14% (8.2% ammoniacal nitrogen + 5.8% nitrate nitrogen).
   Phosphoric acid: 14%.
   Soluble potash: 14%.
   Nutrients are good for 3-4 months at 21 °C.
4. 6 kg calcium carbonate.
5. 655g phosphate (0-18-0).
   Phosphoric acid: 18%.
   Anhydrous calcium sulphate: 48%.
   Combined sulphur (from calcium sulphate) 12%.
6. 22 g fritted trace elements, plant products No. 302.
7. 10 g chelated iron (13%).
8. Washed sand.

Method:

1. Shred all peat moss to a fine uniform texture.
2. Thoroughly mix nutrients (1-6) above into the shredded peat moss.
3. Evenly mix the vermiculite into the peat moss-nutrient mixture.
4. Add 1 part sand to 3 parts of above mixture (1-7) and stir until uniform.